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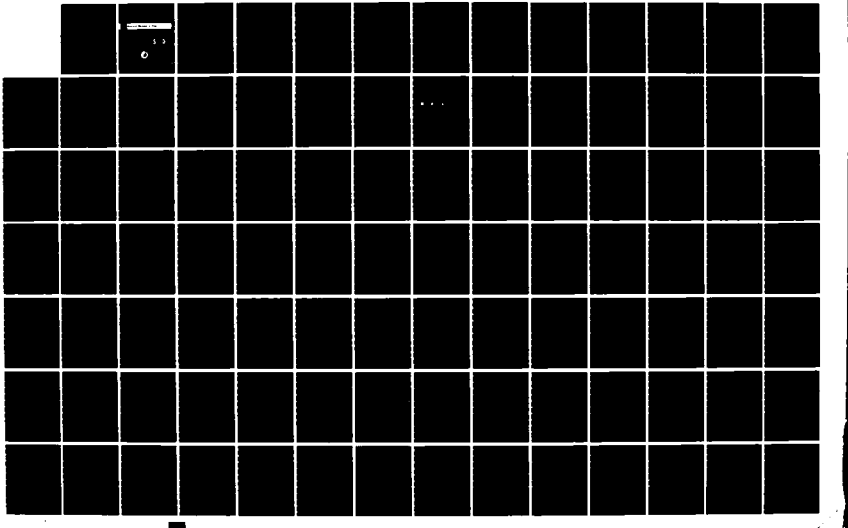

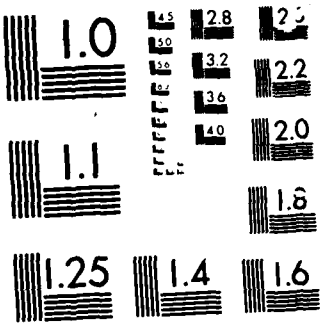

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Annual Research Report

Fiscal Year 1985

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This report reviews the scientific program and describes the status of in-house research at AFRRI for the period 1 October 1984 through 30 September 1985.

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INTRODUCTION

The Armed Forces Radiobiology Research Institute was established in 1961 as a subordinate command of the Defense Nuclear Agency. It is the primary Department of Defense facility for scientific research in the field of radiobiology and related matters. It conducts applied and basic research that is essential for the operational and medical support of the Department of Defense. The work is carried out by five scientific departments as listed below:

Behavioral Sciences: Effects of ionizing radiation, chemicals, and drugs on performance.

Biochemistry: Elucidation of mechanisms of injury, repair, and protection from the effects of ionizing radiation alone or in combination with other agents; development of improved methods to detect and quantify the severity of radiation injury.

Experimental Hematology: Investigation of radiation injury of bone marrow; development of therapy for damage from intermediate radiation doses; determination and treatment of injuries caused by combined effects of radiation, blast, and burns.

Physiology: Research on cellular, tissue, and whole-animal models to determine physiological and biophysical changes resulting from radiation either alone or in combination with drugs or other chemicals.

Radiation Sciences: Operation, maintenance, and quality control of all AFRRI radiation sources; radiation dosimetry and estimation of tissue doses at various depths in different kinds of tissues; development and use of nuclear medicine and magnetic spectroscopic techniques for determining radiation damage in animals and model systems.

The results of this broad multidisciplinary program are summarized in this report. In addition, much of the work is published in the scientific literature, where it contributes significantly to the body of radiobiological knowledge, as well as in AFRRI scientific and technical reports.

REVIEW OF SCIENTIFIC PROGRAM

DOSIMETRY AND TRIAGE

PROBLEM

The assessment of radiation dose received by military forces in the nuclear battlefield has both operational and medical implications. From the viewpoint of the operational commander, it is imperative to know immediately the expected fate of troops who have been irradiated by nuclear detonations. He seeks to answer the following questions:

Can these troops complete their mission?

How soon must replacements be obtained?

Similar questions are posed by the medical commander, but from a slightly different perspective:

Can the life of the patient be saved?

Are the resources available to provide the needed medical care?

The objectives of the research projects presented in this review were (a) to discuss personnel radiation dosimetry, (b) to investigate the role of computers in radiation dosimetry, and (c) to discuss the efficacy of using radiation defects in tooth enamel as dosimeters.

PROGRESS

The projects summarized here have shown significant progress in the last year. Radiation fields produced by nuclear explosions have been recalculated. The low RBE (of 1-3) of neutrons involved in prompt radiation effects (one of the primary concerns of the military) suggests that gamma dosimetry alone may be sufficient on the nuclear battlefield.

Research has been initiated to consider transport calculations using computer simulations of the heterogeneous radiation environment in outer space. Physical and chemical changes induced by gamma radiation in hydroxyapatite have been quantitatively measured. The reliability of using these radiation-induced defects has been addressed. The next several paragraphs summarize in detail the specific progress in these areas.

Personnel Dosimetry in the Nuclear Battlefield (Zeman)

Radiation fields produced by nuclear detonations have been recalculated under a recent DNA-STBE contract with Science Applications, Inc. (SAI). These model radiation fields can and should be used to calculate the expected accuracy of military dosimeters in the battlefield. Similar work has been done in the past by SAI for the German military, using less realistic radiation fields. This type of work should now be repeated, using the more realistic and varied fields available from the recent SAI contract. An inescapable conclusion of recent calculations of

radiation fields produced by nuclear weapon detonations is that the neutron component of total dose is smaller than the gamma component, in some cases much smaller. Indeed, this same result has received high publicity with regard to the dosimetry of Hiroshima and Nagasaki. This finding, plus the relatively low RBE (i.e., 1-3) of neutrons for prompt radiation effects of concern to the military, suggests that gamma dosimetry alone may be sufficient on the nuclear battlefield. This position is supported by the relatively rapid attenuation of neutron radiation in tissue, relative to the attenuation of gamma rays, which causes the neutron component of dose to be even smaller with greater tissue depths. Concepts and policies pertaining to the use of personnel dosimetry in military situations have been surveyed through participation in key meetings. The Triservice RADIAC Conference, a major communication and coordination body between the Services with regard to dosimetry research and hardware, was attended in October 1983 by CDR Zeman. Another meeting is to be scheduled for Spring/Summer 1985. The NATO RSG.5 Group meets every 18 months to set NATC policy on the LD-50, neutron RBE, and personnel and biological dosimetry. AFRRI has played a key role in this NATO panel for several years.

Computerization of Radiation Dosimetry and Space Radiation Measurements (Hsieh)

The progress report in this area is limited since much of the material presented was a proposal for future work. The objective of the proposed project is to establish in-house calculational ability in radiation dosimetry using advanced computer technology. With the availability of the supercomputer at DNA and the minicomputer at AFRRI, we can make the most effective use of in-house resources. At present, the basic 1-D computer code, ANISN (Multigroup One-Dimensional Discrete Ordinate Transport Code System with Anisotropic Scattering), is being installed under the contract with RSIC/ORNL. The original code was written for the IBM-360. The working code will be set up in our computer, VAX-11/750.

Electron Paramagnetic Resonance (EPR) Response of Powder Hydroxyapatite to Low-Energy Electron Irradiation (Swenberg)

Ionizing irradiation of the mineral part of either bones or teeth gives rise to an EPR signal that apparently persists for many years and also color centers. The color centers alter the fluorescence and absorption properties of the material. The observation that the EPR signal increases monotonically with total dose highly suggests that it could serve as a dosimeter. Although numerous studies have been performed, reported results are inconsistent. So a fundamental understanding of the origin of the EPR signal is still lacking. To access the accuracy and sensitivity of the detection of ionizing radiation-induced defects in enamel and any claims as to the possibility of using the induced paramagnetic signal as a biological dosimeter, calculations of quantitative radiation damage have been performed for powder hydroxyapatite. Theoretical calculations have been performed on the relative atomic displacement probabilities for the four different kinds of ions (H, O, P, and Ca) that compose hydroxyapatite, for electrons with energies up to 2 MeV. The fractional probabilities decrease with increasing electron energies for both H and O ions but increase for both Ca and P ions, with each exhibiting slightly

different energy thresholds. Preliminary EPR data indicate that the total signal intensity increases with incident electron energy and that the intensity has a threshold near 0.5 MeV. Initial comparison of experiments with theory identifies the displaced ion with phosphorus if the lattice displacement energy is 25 eV. A preliminary study of the fluorescence from gamma-irradiated whole teeth has also been performed. Irradiated tooth fluorescence emission exhibits a blue shift relative to its nonirradiated control by approximately 10 to 70 A. The slight difference in emission is tentatively attributed to radiation-induced color centers, the molecular nature of which is not known due to insufficient knowledge at the molecular level of fluorescence from tooth enamel.

RECOMMENDATIONS

Experimental work to evaluate the accuracy and reliability of present and future military dosimeters will be contracted to external laboratories due to the nonavailability of manpower within AFRRI.

A second contractual effort will be initiated to calculate the response of present and future military dosimeters in radiation fields produced by nuclear detonations. This contract will include parallel calculation of dosimeter response to selected laboratory radiation fields so that the calculations can be verified by experiment.

Special emphasis will be placed on assessing the importance (or lack of importance) of the neutron component of radiation dose due to nuclear detonations. Specifically, a second contractor (not from SAI) will be sought to verify the accuracy of recent SAI calculations of nuclear-detonation radiation fields, due to the high importance and relevance of these calculations.

Existing military gamma-only dosimeters will be included in contracts to evaluate dosimeter performance in nuclear-detonation radiation fields. The gamma-only dosimeters may prove to be sufficiently accurate for nuclear battlefield applications.

Ionizing radiation is a major hazard in long-term space travel. Computer calculations are required to evaluate and quantitate this hazard. These requirements will include the development of computer simulation capability and Monte Carlo-type calculations. Development of these computer codes will allow quantitation of the spatial-temporal distribution of free-radical production following neutron exposure in a defined environment. It is proposed that AFRRI/DNA possess a library of corporate knowledge to prepare for a possible research program to study the effects of space radiation on humans. It is recommended that at least minimal effort continue in the monitoring of potential problems related to human exposures from space radiations.

Since the current understanding of either radiation-induced changes in enamel fluorescence or paramagnetic centers is poor, the development of a reliable dosimeter based on either of these alterations by ionizing radiation does not appear feasible. Nevertheless, minimal research effort will be maintained, since results could be useful markers in radiation accidents. In such cases, observed physiological changes could be correlated with the quantitative doses received by the victims.

MOLECULAR CELL DAMAGE

PROBLEM

In man, and vertebrates in general, lethal radiation injury, unlike most disease states and other forms of injury, results from physical lesions within the cell. Further, within the cell, physical damage occurs as discrete events at the molecular level. These facts support two rather important corollaries: (a) physiological injury (i.e., noticeable injury) develops only after the organism attempts to metabolize damaged molecules; and (b) any sparing effect must reduce the physical damage or cause the metabolic processes to be more efficient. In mammalian tissues, these metabolic processes are largely unknown. However, the biophysical processes involved in the actual deposition of molecular damage have received a good deal of attention. As a result, we now know that injury at the molecular level is a function of the type of radiation and is quite sensitive to the intracellular milieu, the chemical soup in which these reactions occur. This milieu is in dynamic flux, especially in proliferating cells. As proliferating cells (the most radiosensitive category) proceed through their cycle of replication, the enzymes responsible for repair of radiation damage, the concentration of molecules that scavenge free radicals produced by radiation, and the structure of the radiosensitive target (i.e., DNA) go through large excursions in form and concentration. Ten years ago these variables represented a bewildering ensemble; now each one can be studied and the proliferating cell controlled to address the effects of the constantly changing milieu.

Cellular radiobiology research is specifically organized to address these complexities. With a laboratory devoted to the synchronized culture of proliferating cells, the patterns of change for repair enzymes, DNA structure, and free radical scavengers have become subjects of intense investigation. As a result, the program is separable into three research themes, as follows:

Study of DNA lesions induced by ionizing radiation

Study of the cellular expression and modification of lesions in DNA

Response at the tissue level to radiation injury

PROGRESS

Characterization of Lesions

Hydroxyl Radical Model (Blakely, Hagan, Kumar, Dodgen, Holahan, Weiss)

Damage induced by the irradiation of DNA results in large part from hydroxyl radicals created by the radiolysis of water. The purpose of this study is to create a method for controlled exposure of DNA to chemically produced hydroxyl radicals. The model, planned for development over a period of 3-5 years, will include controlled generation of hydroxyl radicals, quantification of radical yield, characterization of DNA damage resulting from hydroxyl radicals, study of the repair of DNA damage, and correlative studies of this type of DNA damage and cell survival.

Hydrogen peroxide (H_2O_2) has been used to create hydroxyl radicals in solution. Under these conditions, hydroxyl radicals are produced through a Fenton-type reaction involving transition metals that can cycle through two oxidation states. In solution, Mohr's salt is used as a source of ferrous ion, and quantitation is accomplished by observing the production of ethylene gas during the oxidation of methional. Studies involving the exposure of DNA to H_2O_2 have shown that the yield of alkali-labile lesions is high, with millimolar H_2O_2 producing greater than 10 Gy equivalents of DNA damage in 30 min at 0°C. Interestingly, under these same conditions, little cell killing occurs, illustrating an aggressive repair system within the cell. Comparative cell survival studies have shown that the threshold for H_2O_2-induced cell killing increases from ~5.0×10^{-4} to ~5.0×10^{-2} when the temperature during the exposure is reduced from 37°C to 0°C. When this cell killing was investigated through the cell cycle, the S-phase was shown to be the most sensitive period of the cycle, with marked sparing in non-S-phase. A correlation of this pattern of survival with the endogenous levels of glutathione (part of a study in glutathione metabolism discussed below) led to a systematic investigation of the oxidative protective enzymes. To date these studies have involved glutathione peroxidase, catalase, and superoxide dismutase.

DNA Base Damage (Holwitt, Hagan, Dizdaroglu, Blakely, Bergtold, Simic)

The purpose of this work is to exploit the use of selective ion GC-mass spectroscopy to follow the production and removal of DNA bases damaged by ionizing radiation. This newly derived, sensitive technique has been used to observe the spectrum of radiolytic products produced by the gamma irradiation of DNA. The initial goal is to observe the radiolytic production and enzymatic removal of thymine glycol. The glycol of thymine has been recognized and quantified. Also, extensive dialysis has shown that this substrate is not released from DNA in the absence of enzymatic activity.

Effects of DNA Damage on Semiconservative DNA Synthesis (Hagan, Ben-Hur)

It is the purpose of this study to examine the effects of DNA chain breaks on semiconservative synthesis. For mammalian cells, the mechanism of semi-conservative synthesis is poorly understood. However, there is evidence of an association of repair with sites of replication and evidence for specific blockage of initiation or elongation by DNA damage.

By controlling damage in DNA to be behind the replication fork, the effects of "downstream" chain breaks were studied. Surprisingly, chain breaks within 90 minutes of the growing fork halted DNA synthesis. Both initiation and elongation were interrupted. Lesions were restricted within the genome through pulse-labeling synchronously growing cultures. With this technique the DNA damage was limited to 3%-5% of the genome. The labeling, incorporating the thymidine analog bromodeoxyuridine (BrdUrd), provided a convenient source of strand breaks elicited with exposure to 313 nm light. While it is possible to use this technique to investigate prereplicative lesions, this would be technically difficult. An increase in time between BrdUrd incorporation and exposure to 313 nm light allows a degeneration in the quality of the synchrony.

Cellular Expression and Modification of Lesion(s)

Modification of Sulfhydryl Concentration (Holahan, Walden, Blakely, Hagan, Jacocks, Schneiderman)

The purpose of this project is to investigate the link between the level of sulfhydryl compounds within mammalian cells and their inherent radiosensitivity. Sulfhydryl compounds, in particular nonprotein sulfhydryl compounds, can scavenge radicals from solution. This capacity and the apparent correlation of nonprotein sulfhydryls with the variation in radiosensitivity through the cell cycle are widely cited as evidence for a cause-and-effect linkage. To investigate this issue, an HPLC laboratory was established to quantify various nonprotein sulfhydryls at the nanomolar level. Measurements through the cell cycle have been accomplished. From this preliminary work it appears that mitosis is associated with a large increase in the concentration of glutathione, the primary nonprotein sulfhydryl. This aspect of the project is now being confirmed with the use of inhibitors of glutathione synthesis. A second aspect of this project is the correlation of the levels of glutathione in mitosis with cell survival. Here cells are pretreated with drugs that either bind/chelate sulfhydryls or block glutathione synthesis; then the cells are irradiated. The sulfhydryl concentration is measured and observed for correlation with survival.

It has been noted from in vivo studies that prostaglandin E_2 (PGE_2) protects intestinal stem cells from ionizing radiation. PGE_2 may be related to sulfhydryl levels by way of the glutathione-coupled transport of amino acids. We have undertaken to compare PGE_2 effects on cell survival after irradiation with sensitivity to glutathione chelators. Thus far, PGE_2 has been shown to be radioprotective in vitro only when administered for several days before irradiation, or when administered after exposure of cells to the sulfhydryl chelator N-ethylmaleimide. In these two cases, a modest amount of radioprotection was noticed. This radioprotection is only modestly dependent on the age within the cell cycle.

Enzymology of Base Damage Repair (Hagan, Dizdaroglu, Holwitt, Blakely, Holahan)

It has become apparent in the past 4 years that the survival of mammalian cells after an exposure to ionizing radiation is governed strongly by repair processes. Focusing primarily on DNA base damage removal, a systematic approach was developed toward understanding these processes in this relatively poorly understood area. The three areas of approach are (a) identification of radiolytic products of DNA that are removed by cellular enzymes, (b) development of microassays for these enzymes, and (c) study of the regulation of these enzymes through the life cycle of the irradiated cell. Presently, each arm of the study is active.

DNA base damage removable by cellular enzymes is identified as those moieties released from irradiated DNA after exposure to partially purified samples of cellular proteins. To determine products that are released, a careful examination of the initial spectrum of base damage products has been completed. The ensemble of initial radiolytic products is identified through GC-mass spectroscopy.

This procedure is a state-of-the-art quantitative analysis provided by a recently developed technique that provides rapid and accurate identification. Having already identified a previously undiscovered product of guanine, the collaborator for this work, Dr. M. Dizdaroglu (National Bureau of Standards, NBS), has turned his attention to those products available for and removed by cellular extracts. Thus far this work, a collaborative project between AFRRI (Cellular Radiobiology Division) and NBS, has identified the major radiolytic products of DNA in samples of the size and type that will be used in cellular studies at AFRRI. The next milestone for this project will be the quantification of thymine glycol glycosylase activity, which removes the most abundant radiolytic product of thymine. Some current knowledge does exist for thymine glycol glycosylase activity. It is interesting to note that this analytic procedure has identified cytosine glycol as a previously unknown product of OsO_4-treated DNA. It is quite likely that the verification of thymine glycol glycosylase activity will also identify any activity specific for cytosine glycol.

Of the known mammalian enzymes specific for DNA base damage, uracil DNA glycosylase, thymine glycol glycosylase, and endonucleases specific for gamma-irradiated DNA are currently under study. Uracil DNA glycosylase has been identified by the removal of radiolabeled uracil from synthetic DNA. This enzyme activity has now been extensively investigated through the life cycle of the cell, with the following new information. The enzyme activity is regulated strictly at mitosis, where no activity has been detected, and is resynthesized during the time when new DNA is synthesized. The enzyme activity, however, is not coupled to DNA synthesized.

The assay for thymine glycol glycosylase involves both the demonstration of the removal of thymine glycol through chromatographic methods and the observation of the resultant apyrimidinic site. This assay provides a corroboration for the base damage project described above and a microassay for the study of the cellular regulation of this enzyme. At present, thymine glycol has been identified in DNA, synthesized, and purified. The removal of thymine glycol has been indirectly shown through the solubilization of labeled thymine after treatment of DNA with OsO_4. The relative abundance of thymine glycol after exposure of DNA to ionizing radiation makes this activity an obvious first choice for the base damage repair project. In the absence of evidence for specific endonuclease directed toward irradiated DNA, a general assay has been developed for the nicking of irradiated DNA. For this assay, supercoiled viral DNA is exposed to gamma radiation, and cellular endonuclease activity is observed through the relaxation of the supercoiled molecule. Once again this enzyme activity has been shown to be regulated at mitosis.

Repair Patches Formed During the Repair of DNA Damage (Hagan, Blakely, Beer, Koval, Dodgen, Dillehay)

When lesions are removed from parental DNA, enough material is removed to permit resynthesized "patches" to be measured. Routinely, the replacement of thymine with BrdUrd allows the patch to be broken by a subsequent exposure to near ultraviolet (UV) light. Using this technique, we undertook to examine two related sublines of mouse L5178Y cells. Of these two lines, one, designated

L5178Y-R, is resistant to X rays but extremely sensitive to UV light. The other, L5178Y-S, is sensitive to X rays but possesses normal sensitivity to UV light. The specific subject of these studies was the comparison between these two sublines of the number of patches formed during the repair of UV-induced lesions in the DNA. Rodent cells do not remove most UV-induced lesions. It was therefore necessary to improve the patch technique to observe the few observable repair sites. The current status of this project is that several improvements in the technique have allowed the observation of the removal of one lesion per 1.0×10^8 daltons of DNA. With this level of accuracy, the L5178Y-S cells have been shown to effect repair at a rate of 1-2 lesions per 1.0×10^8 daltons per day; after 24 hours, L5178Y-R cells effect no repair and, in fact, no DNA synthesis.

Response of Radiation Injury at the Tissue Level

Control of Cell Proliferation in Hematopoietic Tissue (Hagan, E. Holahan, P. Holahan, MacVittie, Patchen, Ainsworth)

The severity of cell killing in irradiated bone marrow is dependent on the rate of cell division at the time of irradiation. Also, radiation itself can recruit normally dormant marrow stem cells into active cycling, exacerbating the problem. The level of proliferation, both chronically and acutely induced, is in fact a measurement of the radiation injury. The study of induced proliferation has recently been made possible through the development of a conditionally lethal treatment, solely conditional on proliferation. This treatment, the incorporation of BrdUrd and subsequent killing by UV light, has shown that proliferation is induced by sparsely ionizing radiation only after a threshold dose has been achieved. Currently, we are investigating the dependence of this process on the qualitative nature of the radiation. Since particles of high energy and charge deposit energy in concentrated structurally correlated tracks, cells damaged in these lesions are spatially correlated. The study of proliferation induced by HZE particles of various energies may provide information concerning the local nature of the proliferative stimulus. Evidence has been produced that demonstrates the cycling of stem cells in some animals following low-dose irradiation with iron particles at 600 MeV/amu. Currently, argon and lanthanum are also being studied due to their disparate LET's.

Radiobiology of the Regulation of Hexose Transport (Moran, Hagan)

The study of radiation effects on gene expression are difficult in mammalian cells because the time required for induction may be many hours, during which time radiation effects on cell proliferation may dominate. To address this difficulty, plateau-phase cultures of porcine kidney cells were developed. The cultures have very few proliferative cells and are subject to regulating agents that normally affect kidney epithelia.

Currently, we have shown that glucose transport can be induced by lowering the concentration of glucose in the medium to 5 mM. In addition, exposure to ionizing radiation or cyclohexamide blocks induction for several days. The induction block by ionizing radiation is a dose-dependent phenomenon with a D_0 of ~25 Gy, twentyfold higher than that for cell killing. In the past year we have clearly separated the radiation block of induced glucose transport from radiation-induced cell killing.

RECOMMENDATIONS

Characterization of Lesions in DNA

The development of a hydroxyl radical model will take 3-5 years. Three goals have been set for FY 1986: development of the methional reaction to quantitate hydroxyl radical production, identification of specific types of base damage induced by hydroxyl radicals (involves the AFRRI-NBS collaboration), and comparison of the kinetics of lesion repair with cell recovery. Intensive investigation will be ensured to develop this model.

The study of DNA base damage by ionizing radiation will continue. The immediate objective is the development of a reliable assay for thymine glycol glycosylase activity. This study must be continued if we are to observe the radiolytic production and enzymatic removal of thymine glycol and other altered bases formed by ionizing radiation.

Investigations on the effects of DNA damage on semiconservative DNA synthesis are planned to focus on lesions upstream to the replication fork. Although this research is not without technical difficulties, the study of the effect of the prereplicative lesion is the goal for FY 1986.

Cellular Expression and Modification of Lesions

The study of the importance of sulfhydryl compounds within mammalian cells and radiation sensitivity will be extended through FY 1986 to include a characterization of cell cycle effects. Mechanistic studies involving the depletion of intracellular thiols and using synchronous cells are planned. Characterization of the metabolism of exogenously added PGE_2 by V79 cells will be performed in order to compliment previous radioprotective studies.

Several new approaches to the study of enzymology of base damage repair have been initiated. A major goal includes the development of a straightforward chemically based assay for thymine glycol glycosylase activity. The study of the mechanism for cell cycle enzyme regulation and synthesis using this technique or conventional approaches for assaying for glycosylase activity as well as endonuclease and exonuclease activity are current goals for FY 1986. These studies will continue in order to provide a better understanding of repair processes.

During FY 1986, the study of repair patches formed during the repair of DNA damages will be expanded to observe the association of repair with the recovery of DNA synthesis.

Response of Radiation Injury at the Tissue Level

In the study of the control of cell proliferation in hematopoietic tissues, emphasis will be placed in FY 1986 on verifying the Fe data and extending to higher and lower LET's.

For FY 1985 and FY 1986, the study of radiation effects on gene expression will be developed along the hypothesis that inhibited repair of DNA should exacerbate

radiation effects if genetic expression is actually involved. A complete dissection of the molecular biology depends on the isolation of either the gene or gene product. Neither has been done. The study has progressed well, and it will continue.

METABOLIC CELL DAMAGE

PROBLEM

Radiation injury expressed on the behavioral, physiological, and immunological level is a direct result of radiation damage sustained and expressed at the cellular level. Understanding the mechanisms of this cellular radiation injury is an essential first step in the effective management of this injury at the physiological level. It is important to recognize that the immediate incapacitating effects following irradiation are not due to DNA and cell division damage but rather to the results of impairment of normal physiology at the cellular level. Primary radiation insults occurring at the level of the plasma membrane, expressed as alterations in transport phenomena, may result in the release of substances that affect cell function *in situ*.

Several cell systems have been used to investigate these cellular effects of radiation. These include the mast cell, a variety of tissue culture lines, and epithelial cells.

PROGRESS

Monitoring Cytoplasmic Free Calcium in Mast Cells with an Intracellular Fluorescent Indicator (Donlon)

Mast cell degranulation, in response to radiation injury, influences performance decrement and physiological failure, and it may play a role in immunological depression, resulting in compromised recovery of the irradiated individual. Mast cell-stabilizing compounds may prove effective in alleviating these irradiation effects by inhibiting the generation of primary and secondary mediators. These compounds act by altering calcium mobilization in the mast cell. Therefore, an understanding of calcium metabolism in mast cells is an essential step in the development of effective inhibitors of mast cell secretion.

A fluorescent intracellular calcium indicator (Quin-2) has been synthesized, which allows the determination of free calcium concentrations within mast cells following stimulation by a variety of secretagogues. Techniques have been perfected for monitoring alterations in free calcium in mast cells using Quin-2.

Kinetics of Calcium Metabolism and Histamine Release in the Mast Cell (McClain)

The direct effects of radiation on the mast cell *in vitro* have been examined. It does not appear that radiation-stimulated histamine release occurs in the organism as a result of a direct action of radiation on the mast cell. It is probable that radiation leads to the production of agents external to the mast cell, which then interact with the cell to stimulate secretion.

The rapid kinetics of calcium uptake using calcium-45 and its relationship to histamine release has been examined. The bulk of the calcium taken up by the cell as a result of stimulation does not seem to be the trigger for the events leading to

histamine secretion. Instead, calcium uptake appears to occur as a result of secretion. The rise in intracelllular calcium required to trigger secretion is therefore derived from intracellular stores of calcium, not the extracellular medium.

Early Changes in Membrane Function Induced by Radiation (Fuchs)

Ionizing radiation-induced changes on the biophysical and biochemical properties of plasma membranes in cells are similar to those observed in virus-adsorbed cells. Cells irradiated at 250 or 500 rads (doses in which about 40% and 15% of the cells survive, respectively) become leaky immediately following the irradiation. They exhibit a highly elevated efflux rate of 3H-deoxyglucose. This change is transient, lasting about 20-30 minutes; at that time the irradiated cells resume the control rate of efflux. This change is inhibited by the addition of ATP. At the same time there is a transient, markedly increased uptake of fluorescein diacetate into irradiated cells in a dose-dependent manner.

These changes are very similar to those observed with virus-adsorbed cells. Thus, from this point of view, ionizing irradiation effects on plasma membranes can be looked on as some form of ligand binding to the cell.

Spectroscopic Analysis of Intracellular pH and Calcium in Single Cells (Foskett)

Previous work at this and other Institutes has demonstrated that epithelial cells are susceptible to radiation damage. The gastrointestinal syndrome is probably the best example. The problem is to define the loci of this susceptibility. The barrier function of epithelium depends on the transport properties of the cell membranes and on the functional integrity of the tight junction. Radiation damage could be at any of these sites.

Epithelial cells are polarized; the transport properties of the apical membrane differ from those of the basolateral membrane. This asymmetry is necessary for vectoral salt and water transport, and it is maintained by intact tight junctions. Thus, it is desired to study epithelial transport using intact tissues. However, intact tissues are heterogeneous; the properties of the cellular pathway differ from those of the paracellular pathway, and different cell types may have different functions. Thus, in order to assess the effects of radiation on epithelial function, it is necessary to study the intact tissue with techniques that allow analysis of the individual cell types and the tight junctional pathway.

Within this context, it is necessary to define the mechanism of action of radiation effects on these individual pathways. Active transport pathways as well as membrane and tight junctional permeabilities can be controlled by intracellular mediators, including cAMP, CA_{+2}, and pH. It may be that radiation upsets epithelial function via alteration of these intracellular parameters. The goal of this research is to develop and use methodologies to make quantitative measurements of pH and Ca within single cells in intact epithelium.

The Regulator System of Hexose Transport as a Possible Mode for Gene Expression in Mammalian Cells (Moran)

Radiation effects on the regulatory system of hexose transport have been separated from the possible complication of radiation effects on cell division. This

system may provide a functional assay for radiation effects on specific gene expression.

The problem in establishing a model for the effect of radiation on gene expression is to distinguish between (a) the effect of radiation on cell dysfunction caused by changes in specific gene expression and (b) the radiation-induced general impairment of cell division. The ability to separate these two effects has been accomplished by using plateau-phase (nondividing) epithelial cells. This work has shown that the effects of radiation on cell division differ from its effect on the hexose transport regulatory system in the following ways: radiation dose response, kinetics of cell division and of the regulation of hexose transport following irradiation, and response to inhibitors of protein synthesis. This system may serve as a model for radiation effects on specific gene expressions.

De Novo Membrane Generation: Implication of Hydration Forces in Mechanism of Histamine Release (Schmauder-Chock, Chock)

We have made great strides in understanding the mechanism of histamine release from mast cells. This knowledge has enabled us to gain insight into the roles of different molecular forces that govern secretion.

A water influx into the granule of a quiescent mast cell is an early event in the sequence of cellular release. Prior to stimulation, the granule contains high concentrations of preformed mediators in a condensed and osmotically inert state. The condensation of the granule matrix, with concomitant water exclusion, occurs by an unknown mechanism. Reversal of this mechanism, or decondensation, increases the osmolarity, resulting in rapid water influx and matrix swelling. Since granule decondensation occurs in milliseconds, it is an event that is impossible to observe with routine ultrastructural techniques. We have evolved two techniques that have made possible a first glimpse of a cascade of events that culminates in histamine release.

In the first method, rat peritoneal mast cells were rapid-frozen and freeze-substituted before routine ultrastructural observation. The extreme cold used in this procedure spontaneously stimulated and fixed the cells. The second method, which we have named "the SAFME procedure," consisted of the use of saponin to stimulate release in the presence of a fixative (glutaraldehyde) and a membrane image enhancer (tannic acid). This procedure of simultaneous activation, fixation, and membrane enhancement can be abbreviated to SAFME.

Elevation of the perigranular membrane and swelling of the granule matrix are considered as early indications of release (Figure 1). Our results indicate that as the granule matrix begins to decondense coincidentally with a water influx, hydrophobic membrane precursor elements in the matrix periphery are sequestered. The continued water influx causes the sequestered membrane precursor to form a vesicular membrane bilayer, which we referred to as "beads." These beads, which predominate at the periphery of the granule, rapidly fuse with the perigranular membrane, causing it to lift from the surface of the matrix. These patches of newly inserted membrane may be specialized areas in the perigranular membrane that are capable of rapid fusion with the plasma membrane, resulting in pore formation to the exterior. Expansion of the perigranular membrane also increases the probability of its interaction with the membrane of the adjacent granules, promoting the occurrence of compound exocytosis.

The force that enables these events to occur may have accumulated as a result of the removal of intermolecular surface water during granule matrix condensation. This force would require an energy expenditure equal to or greater than that produced by the hydrolysis of ATP. Since hydration force is a direct result of the ionic characteristics of the interacting surfaces, the ionization of these surfaces as caused by ionizing radiation may perturb the ordered state. Disorder would elicit a water influx and initiate the sequence of events that culminates in histamine release.

This study is an observation of the early events in mast cell release, and is the first observation of de novo membrane generation. The forces that bring about these changes, whether initiated by receptor-mediated release or ionizing radiation, exploit the mechanism of hydration forces.

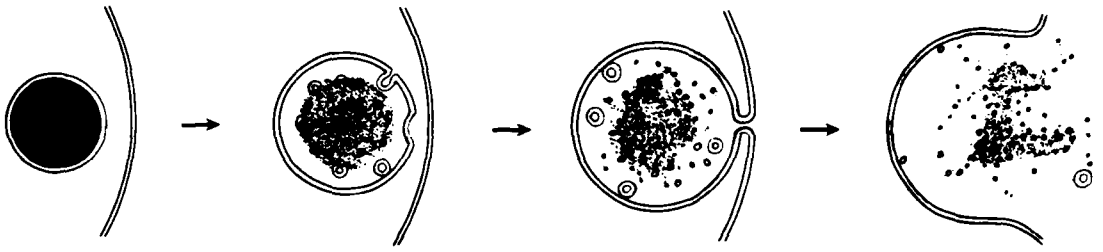

Fig. 1. Scheme of two-stage fusion model for secretion. Activation of condensed quiescent granule leads to process of decondensation. During early part of decondensation, bilayer beads are rapidly generated and inserted into perigranular membrane. This fusion between the newly formed beads and the perigranular membrane represents first fusion event. This results in lifting and expansion of perigranular membrane. Fusion between expanding perigranular membrane and plasma membrane, which represents second fusion event, results in pore formation and extrusion of granule matrix and its contents.

RECOMMENDATIONS

Investigations will continue on calcium mobilization during histamine release, initially using the Quin-2 probe. Several newly developed antianaphylactic compounds and selective calcium antagonists will be studied for their effects on calcium mobilization in the mast cell. A variety of secretagogues will be examined. A newly synthesized fluorescent intracellular calcium indicator, Fura-2, which offers several advantages over Quin-2, will be examined in this experimental system. In addition to the precise quantitation of intracellular calcium changes occurring during secretion, this system will also provide for the rapid screening of newly synthesized mast cell-stabilizing compounds. These compounds can be further tested in radiation/combined-injury animal models to determine their efficacy in modulating function, recovery, and survival.

The examination of radiation-induced early changes in the membranes of cultured cells will continue the study of changes in transport mechanisms following irradiation. Additional studies will include radiation-induced changes in the physical state of the membrane (membrane fluidity), the function of cellular receptors (for viruses or drugs) following irradiation, and the effects of radioprotectants on the altered membrane functions.

Investigations involving an optical assessment of pH and calcium changes at the cellular level will be strongly supported. The advantages of an optical approach using fluorescence are (a) the ability to record rapid transients, (b) the high sensitivity and generally high signal/noise ratio, (c) the ability to obtain spatial information so that different cell types and different parts of single cells can be analyzed, (d) the ability to use an intact tissue, and (e) the fact that dye leakage is not a problem (as a result of using perfusion techniques), as it is in cuvette-based measurements. The specific approach will use emission spectroscopy, in which the entire spectrum is captured simultaneously, using a television tube as a multichannel detector. This approach permits use of ratioing techniques, making quantitation independent of (a) dye concentration (thus nonuniform loading of the dye or dye leakage is unimportant), (b) dye bleaching, and (c) intensity fluctuations of the light source. Emission spectroscopy permits the detection of light scattering and binding effects as well as chemical modification of the probe. If these effects are not accounted for (i.e., by measuring only one emission wavelength), they could lead to spurious quantification.

Refinement of the hexose transport model for radiation effects on specific gene expressions is required. A technique to quantitatively define the number of included proteins is also required. The role of DNA in the observed changes in hexose transport will be examined by modulating the DNA repair mechanisms in the cells.

GASTROINTESTINAL INJURY

PROBLEM

Exposure to ionizing radiation produces considerable gastrointestinal dysfunction. Doses as low as 1 Gy elicit prodromal effects characterized by nausea, vomiting, and diarrhea. Although these effects are not life-threatening, they do lead to severe incapacitation. Doses of gamma radiation greater than 10 Gy and lower doses of neutron exposure (approximately 3 Gy) elicit the gastrointestinal syndrome and its associated fluid and electrolyte losses that do result in radiation lethality at these doses. Considering the broad implications of radiation-induced gastrointestinal injury, studies on the mechanisms underlying this pathophysiology and methods to mitigate these changes are necessary to prolong survival and to attenuate incapacitation.

PROGRESS

During the past year, substantial progress was made in the management of radiation-induced gastrointestinal injury, by defining the magnitude of the dysfunction and by evaluating potential techniques of mitigating the dysfunction. During the 1985 AFRRI Program Review, progress reports were given for six studies encompassing prodromal effects and the gastrointestinal syndrome, as follows:

Alterations of gastric function accompanying the prodromal effects

Effect of irradiation on hepatobiliary function

Mechanisms of radiation damage and radioprotection in the gastrointestinal system

Biochemical indicators of radiation-induced gastrointestinal injury

Physiological mechanism of acute intestinal radiation

Radiation-induced changes in transport processes

The diversity of these studies indicates the complexity of radiation's effects on gastrointestinal function.

Alterations of Gastric Function Accompanying the Prodromal Effects (Dubois)

Using a subhuman primate model, the possible role of prostaglandins in radiation's suppression of gastric emptying was investigated. In monkeys irradiated with 800 cGy whole-body gamma, gastric juice levels but not plasma levels of the prostaglandins (PG) PGE_2 and PGE_{12} were observed to increase immediately and then decline to control values by 2 days postexposure. The time course of these changes was similar to that observed for the suppressed gastric-acid output that was observed following irradiation. Gastric $PGF_{2\alpha}$ levels, however, did not increase until 2 days following irradiation. These results suggest that PGE_2 and PGE_{12} may

be responsible for the immediate suppression of acid output while $PGF_{2\alpha}$ may play a role in delayed radiation sickness. In a different group of experiments, the relation between radiation-induced vomiting, gastric emptying, and plasma β-endorphin levels were investigated by evaluating the antiemetic properties of dazopride (AHR5531) in monkeys irradiated with 800 cGy whole-body gamma. Radiation exposure abolished the gastric emptying of both liquids and solids. In animals receiving dazopride before exposure, gastric emptying of solids was not suppressed, and radiation-induced emesis was absent. The increase in plasma β-endorphin following irradiation was not affected by the drug, thus suggesting that dazopride action differs from antiemetic compounds that block dopamine receptors and stimulate the release of β-endorphin.

Effects of Irradiation on Hepatobiliary Function (Durakovic)

The purpose of this study is to determine hepatobiliary kinetics in dogs after whole-body gamma irradiation in an effort to determine if increased biliary output and/or emptying of the gallbladder is a mechanism (via the enteric gastric reflex) for radiation-induced emesis. The intrahepatic and gallbladder transit of bile salts and the evacuation of bile salts into the small intestine were monitored in cobalt-60-irradiated male beagle dogs injected with Tc-99m DISIDA, a biliary homologue, using scintigraphic studies. In animals irradiated with 4 or 8 Gy, no significant changes were seen in tracer visualization of the gallbladder, or in the peak activity of the gallbladder or the liver at 1 and 7 days postexposure, compared to baseline values. In contrast, in the animals irradiated with 8 Gy cobalt-60, intestinal visualization occurred significantly earlier on day 1 and on day 7. Thus, gallbladder filling does not appear to be affected by radiation exposure, whereas emptying is significantly accelerated after 8 Gy. These results suggest that irradiation stimulates gallbladder contractility without modifying intrahepatic kinetics.

Mechanisms of Radiation Damage and Radioprotection in the Gastrointestinal System (Batzri)

The goal of this project is to develop a reliable model system in which to study radiation-induced changes in gastric secretory patterns and their relation to radioemesis. Studies of gastric secretion will be performed both in vivo and in vitro. In vivo studies will use an awake guinea-pig model and the marker dilution technique. In vitro studies will include determining the effect of radiation on isolated gastric glands and mucosal cells and on mucous cells in culture. In addition, the efficacy of radioprotective compounds will be determined, and the mechanisms underlying radiation-induced changes will be explored.

Biochemical Indicators of Radiation-induced Gastrointestinal Injury (Snyder)

These studies indicate that decreases in plasma and ileal tissue levels of diamine oxidase (DAO), an enzyme found in mature intestinal epithelial cells, parallel intestinal radiation damage. Thus, plasma DAO levels may provide a useful marker for the clinical evaluation of radiation-induced gastrointestinal damage and the efficacy of measures designed to minimize the damage in humans.

Physiological Mechanism of Acute Intestinal Radiation (Geraci)

An understanding of the physiological mechanisms that culminate in intestinal death from radiation is essential to the development of medical treatment for intestinal injury. To this end, the study investigates in a rat model the relative contributions of previously proposed mechanisms of intestinal death to radiation lethality. The mechanisms evaluated include (a) fluid and electrolyte losses via the radiation-damaged intestine; (b) action of bile acids on the radiation-damaged intestinal mucosa, causing fluid and electrolyte losses; and (c) escape of enteric bacterial organisms or their endotoxins, causing systemic sepsis or endotoxemia. The results indicate that, of the above, the inability of the intestinal mucosa to absorb or reabsorb fluid and electrolytes is the important physiologic mechanism of intestinal radiation death. At doses of radiation exposure in which intestinal and hematopoietic injury overlap however, the role of enteric bacteria may be significant.

Radiation-induced Changes in Transport Processes (Gunter-Smith)

The goal of the study is to determine the mechanism underlying the radiation-induced fluid and electrolyte losses, which appear to be the major determinant of intestinal radiation death. The study uses a rabbit model and in vitro techniques to measure electrolyte transport in intestinal segments isolated from animals exposed to cobalt-60 radiation. The results indicate significant alterations in cellular transport processes of both the ileum and the colon. Decreased absorptive capacity was observed in the ileum, whereas there appeared to be a compensatory increase in the absorptive capacity of the colon. The dose dependency and time course of these changes have also been determined, and the results suggest that they are related to fluid and electrolyte loss associated with the gastrointestinal syndrome. In conjunction with these studies, the role of punitive intracellular regulators of cell membrane transport processes involved in secretory phenomena such as Ca have also been investigated using electrophysiologic techniques. To this end, agents that alter intracellular Ca levels, such as Ca channel blockers, have been shown to alter the membrane permeability of guinea pig gallbladder epithelial cells (a relatively simple epithelium that has both absorptive and secretory processes similar to ileum).

RECOMMENDATIONS

As indicated by the scope of the above summaries, significant progress has been made in studies concerning radiation-induced alterations of intestinal function. In the area of the prodromal effects, the basic alterations resulting in nausea and vomiting have been probed in greater detail. A possible relation between radiation's suppression of gastric acid secretion and prostaglandin increases has been demonstrated, and the role of β-endorphins in radiation emesis has been evaluated. The search for effective antiradioemetics that lack undesirable side effects continues with the evaluation of antiemetic compounds such as dazopride. In addition, new and more reliable model systems to study radiation-induced pathophysiology are being sought. The proposed use of isolated gastric cells and glands will help determine the locus of radiation damage at the cellular level and help define the role of released mediators in gastrointestinal dysfunction. Furthermore, the ability to correlate data from these in vitro experiments with those of in vivo experiments using the same animal species will prove valuable.

Significant progress has also been made in quantifying the extent of the gastrointestinal syndrome and determining the mechanisms underlying its lethality. Plasma levels of diamine oxidase have been demonstrated to reflect radiation-induced gastrointestinal injury. If these results prove applicable to humans, the assay may be clinically useful and aid physicians in determining a course of treatment. The major mechanism of intestinal radiation death has now been determined after years of controversy. Lethality appears to be due to a decrease in the absorptive capacity of the small intestine. However, fluid and electrolyte losses and bacteremia may contribute to lethality in cases in which intestinal damage and hematopoietic damage are of major consequence. These studies point to the need for investigating ways to optimize fluid and electrolyte therapy and to increase intestinal cell proliferation. In addition, the effectiveness of a combination of treatments for intestinal damage and hematopoietic damage needs to be developed. Recent experiments have demonstrated that the decrease in absorptive capacity noted above results at least in part from radiation's alteration of cell membrane transport processes. Understanding how these changes occur and distinguishing the direct effects of radiation from the indirect effects due to radiation-released agents will be essential to developing successful treatment regimens. Studies determining the effect of radiation on intestinal epithelial cells in culture will be of use. Finally, we need to assess the functional changes in critical end organs (such as the lung, kidney, and liver) that may threaten long-term survival and the quality of survival following recovery from gastrointestinal dysfunction.

CARDIOVASCULAR DYSFUNCTION

PROBLEM

After irradiation, cardiovascular function may be a major determinant of continued performance, if not survival. Study of the etiology of radiation-induced hypotension and the effect that it might have on performance has been hindered by the lack of an adequate experimental model. The aim of this research has been to develop a reliable animal model for studying cardiovascular dysfunction (CVD), delve into the etiology of CVD, and attempt to influence the sequelae of CVD.

PROGRESS

Biochemical and Physiological Changes of Radiation-Induced Cardiovascular Dysfunction (Hawkins and Durakovic)

A model, using chloralose-anesthetized rhesus monkeys, has been developed in which postirradiation cardiovascular dysfunction can be produced in a repeatable and predictable fashion. Using this model, changes in cardiovascular function following whole-body exposure to ionizing radiation have been studied in considerable detail. It was shown that irradiation produces a marked decrease in arterial pressure of 50% within 5 minutes after exposure to 4 krads of 1.2 MeV gamma radiation.

An initial and immediate hypotensive phase appears to be due to a transient reduction in total peripheral resistance (TPR) along with decreased venous return. Occurrence of these responses seems to be due to histamine (a tenfold to fifteenfold increase), which is released immediately after exposure. Then a secondary phase of hypotension occurs (>45 minutes postirradiation), which is associated with a sustained reduction in central venous pressure (CVP) and cardiac index (CI), whereas TPR and plasma histamine levels return to near baseline values.

In an effort to improve postirradiation CI, other monkeys were similarly prepared and irradiated. The application of 80 to 100 mm Hg of positive pressure to the legs and abdomen (lower body positive pressure, LBPP) at 3 minutes postexposure raises arterial pressure above that recorded in nontreated but irradiated animals. Associated with the application of LBPP is a rapid return of CVP to near preexposure values, whereas TPR rises to values above those previously recorded for nontreated but irradiated animals. These rapid changes occur despite a change in plasma histamine values comparable to those described previously. Despite these improvements in pressure and resistance, the application of LBPP did not improve CI above that reported in nontreated irradiated animals.

To determine the effects of hydrostatic pressure on CVD, three animals were prepared in a similar fashion and seated in a chair inclined 60% from horizontal. Exposure to the same level of radiation resulted in hypotension somewhat greater than that observed in supine subjects. Elevation resulted in more severe decreases in CVP and CI than those recorded in the supine subjects, whereas TPR depression was comparable. The application of LBPP to these seated animals 7 minutes before irradiation reduced the magnitude of the initial hypotension response to irradiation, from 60% (recorded in seated subjects without LBPP) to 37%. The

depression in CI was also reduced by the application of LBPP. Both groups experienced similar increases in plasma histamine. In addition, animals exposed in the seated position experienced greater overall difficulties during the 90-minute postexposure observation period than animals in the supine position. Three of the four LBPP animals succumbed between 60 and 90 minutes postexposure. None of the animals exposed in the supine position, with or without LBPP, succumbed within the 90-minute observation period. Despite these improvements in cardiovascular function by the use of LBPP, caution is required because of the effects of LBPP on arterial oxygenation. LBPP, in both supine and seated monkeys, reduced arterial PO_2 from baseline values of 74 and 85 mm Hg to 55 and 63 mm Hg, respectively, within 10 minutes postexposure.

To determine the effects of CVD on major organ perfusion in this model, other chloralose-anesthetized rhesus (n = 7) were prepared for microsphere (15 microns) injections and for the recording of cardiovascular parameters. Within 5 minutes after whole-body exposure to 4 krads, MAP and CI decreased by 58% and 51%, respectively. At this time, percent decreases in regional blood flows were epicardium 39, endocardium 51, cerebrum 43, hypothalamus 64, medulla 61, ileum 26, spleen 93, kidney 45, adrenal 27, and pancreas 60. By 90 minutes postexposure, MAP and CI recovered to 73% and 59% of their respective baselines. Perfusion of heart, brain, and most other organs remained depressed, but ileum and adrenal flows increased above baseline. These data suggest that the extended postexposure hypotension may contribute to hypoperfusion in the brain and other organs. The above-baseline flow to the ileum, in the face of this protracted hypotension, suggests regulatory failure in this organ, which may contribute to subsequent decreases in blood volume.

RECOMMENDATIONS

At this time, no cardiovacular dysfunction program exists because of lack of personnel. Further investigation into the etiology of CVD will be carried out using both neutron and gamma radiation in this model. These studies will concentrate on changes in the systemic circulation that may contribute to postirradiation decreases in circulating blood volume and venous return. In addition, a similar model will be used to investigate and quantitate changes in cardiovascular reserve after paralethal doses of both neutron and gamma irradiation.

COMBINED-INJURY LESIONS

PROBLEM

Nuclear detonation could result in massive numbers of casualties from single or combined (multiple) injuries. Some progress has been made in understanding the underlying biomedical disturbances and their treatment in single-injury individuals. However, only recently has attention been directed toward understanding the basic biomedical disturbances and therapies required for the combined-injury host. Identifying critical derangements in and providing treatments for combined injury is difficult due to the synergism of radiation and trauma effects. That is, sublethal radiation combined with sublethal wound/burn results in profound damage and suppression of the host's defense systems, culminating in death from opportunistic microbes. Strategies aimed at treatment of combined injuries are difficult because no reliable animal models exist for determining underlying humoral and cellular immune and myeloid dysfunctions or for evaluating therapies. The current combined-injury program has a threefold thrust, as follows:

Develop appropriate small-animal and large-animal models of single injury and combined injury for study.

Define the critical derangements produced by single and combined injuries that can be advantageously manipulated, replaced, or repaired.

Evaluate treatment modalities that could result in mission accomplishment and survival.

PROGRESS

Hematologic and Host Resistance, and Immunity

The nonspecific inflammatory and specific cell-mediated immune responses are essential elements of the host's inflammatory and immunological defense against microorganisms. Radiation, trauma, and sepsis can (dose- and intensity-dependent) significantly affect neutrophil and lymphocyte function and the host's ability to resist infection. The integrity of the nonspecific inflammatory response and components of cell-mediated immunity was evaluated by measuring neutrophil function and lymphocyte blastogenesis. Neutrophil migration and bactericidal activity were markedly enhanced in septic animals, while in irradiated animals the activity was depressed or unchanged. The risk for developing an infection and sepsis following irradiation may be related to an impaired ability of the remaining neutrophils to migrate to the site of the infection rather than the loss of the ability to kill the microorganisms. Irradiation transiently depressed lymphocyte blastogenesis and induced cyclic shifts in lymphocyte subpopulations. In contrast, E. coli produced a generalized depression in lymphocyte responsiveness that persisted for several weeks.

Hemodynamics in Canine Sepsis (Natanson, MacVittie, Walker, Conklin)

Endotoxin shock in dogs results in a profound decrease in cardiovascular performance that is unlike the hemodynamic profile of septic shock seen in humans. Humans with septic shock develop a reversible decrease in radionuclide-determined left ventricular ejection fraction, although the cardiac index does not decrease. The canine was used to determine if this reversible decrease occurred, identify

the mechanism of induction, and evaluate therapies. E. coli bacteremia was produced by implanting an infected clot peritoneally. A bacterial concentration dose-response curve was generated ranging from 7 to 30 x 10^9 E. coli per kg of body weight. Baseline and daily cardiac function was evaluated using radionuclide blood pool scans and pulmonary catheters in awake animals under local anesthesia. The dose of bacteria was correlated with the magnitude of myocardial depression. E. coli, 30 x 10^9 per kg of body weight, resulted in a 60% decrease in left ventricular ejection fraction within 48 hours after implant of the bacteria. The ejection fraction increased to baseline by days 7 through 11 postimplant. Additional data indicated a significant decrease in ventricular function and marked evelation in cardiac index, a pattern typical of early human septic shock.

This study suggests that (a) the number of organisms at a local nidus of infection may be an important variable affecting the amount of cardiovascular dysfunction in Gram-negative sepsis, and (b) the canine sepsis model is a valid one for extrapolation to the human septic shock condition. This validity is based on three independent methods of evaluating systemic function: ejection fraction, end diastolic volume index versus left ventricular stroke work, and vector analysis.

Survival From Single or Combined Injury (Ledney, Brook)

No consistent small-animal model exists for evaluating protocols in the determination of therapy modalities that could lead to recovery for combined-injury casualties after nuclear detonation. Further, there is a need to define the pathophysiologic derangements produced after injury, to determine if responses to wounding are the same as or different from responses to burning, for the single injury and for the combined injury.

Mice were subjected to 30%-body-surface skin wounding or to 30%-body-surface skin burns, either alone or at various times before or after a sublethal dose of 3-Gy fission neutrons. Heart blood and tissues were cultured for bacteria, and mortality was recorded over 30 days. The following results were obtained:

> Five percent mortality resulted from single-burn, wound, or radiation injury.
>
> Mortality rate depended on the type of injury and the timing of injury relative to radiation. The incidence of mortality was additive or synergistic, depending on the combination of timing and injury.
>
> Lethality incidences were greater in wound-radiation combinations than in burn-radiation combinations. Death was associated with presence of Proteus, E. coli, Staphylococcus, and Streptococcus.

Pathophysiologic and therapy modality studies should be evaluated in mice wounded or burned shortly after exposure to sublethal fission neutrons. Mortality of 40% and 15% was found consistently for mice wounded after 3 Gy or burned after 3 Gy, respectively.

Acute-Phase Proteins (Ledney, Gelston)

C-reactive protein (C-RP), an acute-phase protein found as a normal serum constituent, may indicate the severity of trauma, signal early undetectable infections, and modulate mononuclear responses. Monitoring this substance may

indicate the outcome of the treated or nontreated single-injury or combined-injury individual. Groups of mice were subjected to six model situations: normal nontreated, normal wounded, normal burned, irradiated, irradiated-wounded, and irradiated-burned. The serum was obtained from individual mice over a 30-day period and analyzed for C-RP via laser nephelometry. Results indicated that C-RP responses depended on radiation dose, increased early after wounding, and decreased after burn trauma. In combined-injury combinations, radiation depressed the wound, enhanced C-RP levels, and reversed the burn-induced C-RP repression.

Opsonic Factors and C3 (Ledney)

Opsonins are macromolecular substances found in the serum that assist the cellular defenses of the host against infection. The serum concentrations of opsonins in the mouse after single or combined injury are unknown. Differences in opsonic proteins induced by radiation wounding versus radiation burning could explain the mortality incidences (40% versus 15%, respectively) previously noted. Groups of mice were subjected to each of the six test (model) situations previously listed. The serum was obtained from individual mice over a 30-day period and analyzed for the opsonins IgM, IgG, and IgA by laser nephelometry. C3, a serum complement component involved in anaphylotoxin production, was also measured. Opsonic factors were severely reduced after tissue trauma, but they returned to control levels 10 days after injury. IgG was the only opsonin significantly reduced early after irradiation. IgM and IgA were significantly reduced 1 week after exposure. Combined injury produced additive reductions in opsonin levels. No distinct differences were found in opsonins in each combined-injury model. Thus, mortality differences between radiation-wound versus radiation-burn were not explained by differences in opsonic values. C3 was increased after all injuries. The magnitude of increase was greatest after combined injury (similar in each model), and less severely increased after each tissue trauma. There were no differences after burning or wounding in C3 levels. Irradiation produced a modest 5-day increase in C3. Greater mortality in radiation-wound versus radiation-burn was not explained by increased anaphylotoxin levels since similar levels were observed in each combined-injury model.

Circulating Mediators (Ledney, Steel)

Numerous pathobiologic events occurring in injury may be attributed to the actions of chemical mediators. These mediators may act as modulators of the inflammatory response or as necessary components for the generation of inflammation. The circulatory factors and hormonal mediators that may have causal roles in the sequelae of radiation and/or combined-injury performance decrement and incapacitation were investigated. The objectives included (a) determination of trauma-induced alterations in mediator content, and (b) correlation of the kinetics of tissue, plasma, and urinary levels of mediators in trauma with the cellular disturbances produced in the hematopoietic system. The biological test systems included invasive sampling (blood) and noninvasive sampling (urine) from the mouse and guinea pig. Since each mediator is only one element of a complex system of regulatory mechanisms, several with synergistic and/or modulatory relationships were analyzed. These included prostaglandins, thromboxanes, leukotrienes, cyclic nucleotides, complement, ACTH, and cortisol/corticosterone.

Sequential animal testing established a time course of mediator kinetics associated with the development of the radiation injury examined. The role of the classic pathway of complement activation was examined using C4 genetically deficient

guinea pigs. A fundamental role for the classical pathway in survival from acute radiation exposure was not found; however, complement may participate in the immunoregulation of arachidonic acid metabolism/excretion in the radiation-damaged kidney. In the mouse model, the reliability of laboratory measurement of mediators as indicators of radiation insult suggests that the concentration of urinary PGE_2 may be used in the estimation of whole-body radiation exposure levels, over a wide range of radiation doses. The kinetics of the circulating PGE_2 levels in radiation, trauma, or combined injury also implicates this mediator as a potential candidate as modulator (whether direct or indirect) of the immunological responses.

In Vitro and In Vivo Responses of Macrophages (Gallin, Bowers, Patchen, D'Alesandro)

Tissue phagocytes play a pivotal role in host defense by ingesting and killing invading microorganisms and by releasing a variety of inflammatory factors. These cells (macrophages or macrophage-like cells) are found in a variety of organs including the liver, where they are called Kupffer cells. Conflicting reports in the literature describe either activation, inhibition, or no effect of radiation on tissue phagocytes. It is important to differentiate the changes in phagocyte function that occur in a severely compromised animal after whole-body irradiation (in vivo models) from those that occur in a controlled in vitro system. In vitro model systems were used to assess the direct effects of radiation on primary cultures of mouse macrophages and on macrophage-like cell lines. Macrophages were maintained in tissue culture for up to 2 weeks following exposure to 2.5 to 10 Gy of ^{60}Co. At varying times postirradiation, functional and biophysical assays were performed. These included phagocytic, chemotactic, and secretory assays, as well as measurements of ionic conductances in control and irradiated cells. Changes in the free calcium levels in the macrophage have been monitored by following the activation of calcium-sensitive conductances.

Liver macrophage (Kupffer cell) function was assessed in an in vivo rat system. Both irradiation and the administration of exogenous endotoxin increase the state of activation of these cells. Since endotoxin levels usually increase following irradiation, some of the effects of radiation on the Kupffer cells may be due to endotoxin. P-glucan, an immunomodulating agent, also increases the activity of Kupffer cells, and it can serve as a useful model of Kupffer cell activation. Prior treatment of animals with P-glucan enhanced sensitivity to both radiation and endotoxins, supporting the view that activation of the Kupffer cell leads to tissue damage and increased mortality.

In Vitro: Radiation decreases Fc-mediated phagocytosis in primary cultures of mouse peritoneal macrophages in a time-dependent manner. The phagocytic deficit may play a role in the increased susceptibility to infection in irradiated animals. In contrast, the mouse-derived macrophage-like cell line was activated in a time- and dose-dependent manner after irradiation. In these cells, radiation provides a useful tool for comparing a relatively homogenous group of macrophages in two different states of activation.

In Vivo: Treatment with radiation, endotoxin, or P-glucan increased the state of activation of liver Kupffer cells as assessed by the release of thromboxane A_2 and prostaglandin E_2. Inflammatory products released from Kupffer cells likely produce much of the organ damage associated with radiation exposure.

Immunologic Enhancements (Neta, Schwartz)

Single or combined injury results in a condition of severe immunocompromise. Infections resulting in death may ensue in compromised hosts, who under normal situations can cope with the normal bacterial flora. Treatments are needed to support the immunocompromised host infected with "conditioned" or opportunistic pathogens. The ability of daily injections of thymosin fraction 5 (TF5) to repair critical effects of radiation was examined in mice. The survival of irradiated and Pseudomonas aeruginosa-infected mice was measured in TF5-treated mice versus controls, or those irradiated and given saline injections, or those irradiated only. The repopulation of the thymus following radiation was measured in control animals and in animals given TF5. TF5 administered to mice after radiation increased the survival of P. aeruginosa-infected mice. Thus, thymic hormones may be useful in protecting irradiated hosts from opportunistic infections. Treatment with TF5 resulted in recovery of thymic cell populations, with responses to IL-1 higher than normal (nonirradiated) animals. Cells repopulating the thymus in irradiated nontreated animals were not responsive to IL-1. The effects of TF5 on thymic populations are due to either an increase in thymic cell maturation or an increase in influx of mature cells from the bone marrow. Thus, thymic hormones may be important in the recovery of immune function in an irradiated host.

Mucosal Lymphocytes (Hale)

Radiation damages the proliferating crypt elements of the intestine, which, along with the mature cells of the villus, provide a barrier to bacterial infections emanating from the gut lumen. The lymphoid apparatus of the intestinal tract aids the host in protecting against bacteria that penetrate the intestinal epithelial cell layer. These lymphoid elements are also sensitive to radiation, but little is known about the quantitative nature of the damage and the means for enhancing immune cell recovery. Rats were exposed to 1.5 Gy of ^{60}Co at 40 cGy/min. On days 1-20 after irradiation, lymphocytes from the Peyer's patches were analyzed using a fluorescence-activated cell sorter (FACS-II). The percentage of each lymphocyte subpopulation was analyzed. The number of lymphocytes in Peyer's patches decreased following irradiation. The regeneration of Peyer's patch lymphocytes was slower than the regeneration of lymphocytes in other lymphoid organs. Although their regeneration was slower, once accomplished, the repopulated lymphocytes functioned normally. To investigate ways to enhance the rate of regeneration of the mucosal immune system, a chimeric rat model is being developed. Using this system, the effects of radiation on the regeneration of mucosal lymphocytes from bone marrow stem cells can be assessed.

Infection in Irradiated Combined-Injury Mice (Brook, Patchen, Neta, Ledney)

Work attempted to establish the baseline of spontaneous infection for future studies, documenting the importance of endogenous flora as a cause of infection. Also developed was an animal model that simulates human wound and systemic infection after trauma and irradiation with known pathogens. Attempts were made to devise modes of therapy that include antimicrobial agents and immunotherapy (gamma globulin, thymosin, and glucan). Mice (HeN and C_3H) were irradiated with varying amounts of cobalt (7 to 10 Gy). Cultures of blood, liver, spleen, and local lesions were made. A local wound was induced by incising the right glutal area, and different bacterial pathogens were injected into the lesion (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, and Group A streptococci). For experiments involving systemic infection,

P. aeruginosa was fed to mice, and systemic infection was monitored. The bacterial species important in postinoculation sepsis were identified (E. coli, S. aureus, anaerobic cocci, and Bacteroides species). A relationship was found between the radiation dose and the types of bacteria (increased irradiation directly related to anaerobic sepsis). A local wound model was established for the study of therapeutic modalities. Systemic P. aeruginosa infections in irradiated mice were developed, and protection was documented with thymosin, aminoglycosides, and gamma globulin alone or in combination.

RECOMMENDATIONS

The canine model of radiation-induced injury and Gram-negative sepsis will be used to further evaluate the effects of these stresses on hemopoiesis, inflammatory response, cell-mediated immunity, and cardiovascular hemodynamics. During FY 1986, specific projects will include (a) inducing a low percentage of lethality over the first week postimplant of bacteria in the radiation-induced neutropenic host, (b) a mechanistic approach to cardiovascular and neutrophil dysfunctions, and (c) evaluation of the role of extensive fluid therapy after sepsis and combined modality treatment with antibiotics.

The nuclear disaster environment will comprise a radiation field of essentially equal parts of photons and neutrons (an N/G ratio of 1:1). Thus, extension of the current mouse models from an N/G ratio of 25:1 to the anticipated 1:1 ratio is expected. Combined-injury investigations will also include studies using ^{60}Co-produced photons. Once accomplished, the contribution of each type of radiation energy to the mortality and deficiencies in humoral immunity, cellular immunity, and myeloid activity can be assessed. Each situation will be evaluated as to the mortality from nosocomial infections and intentional infections of interest in trauma or mass-casualty situations. These include E. coli, P. aeruginosa, K. pneumoniae, Streptococcus, Staphylococcus, and Proteus. Combination infections will be investigated, including selected Gram-positive and Gram-negative organisms and selected anaerobes and aerobes.

Investigations using rats will be expanded. The larger rodent is adequate for studies in hemorrhagic shock, surgical injury, and wound trauma. Wound trauma studies and combined radiation-wound trauma studies on changes in lymphocyte population of Peyer's patch in the rat will take advantage of similar information that is available for sublethal radiation injury in this species. A bone marrow cell chimera is planned to study the repopulation dynamics of Peyer's patch cells after irradiation. Also, the rat's size permits sequential monitoring of blood and fluid parameters after injury.

Identification of derangements, including quantification, similarities, and differences between each single injury and combined injury, will continue in current and newly developed animal models. Evaluation will focus on the lymphoid and myeloid humoral and cellular compartments. The research array will include in vitro and in vivo cellular, organ, and whole-animal investigations, as follows:

> In vitro investigations with macrophage cell lines and primary cultured mouse macrophages will be used to examine ways of modulating radiation-induced activation. Monitoring of intracellular free calcium and ionic conditions after irradiation will be pursued.

Complement components C3 and C5 are involved in generation of the anaphylotoxins C3a and C5a, which can mediate inflammation via histamine release. The long-term increase of C3 after all single and combined injuries in mice suggests that anaphylotoxins may be generated and involved in the pathophysiologic events that lead to death. These factors plus the increases of PGE_2 in all injury situations may be the principal lesions that lead to infectious complications and death in mice subjected to single and combined injuries. C3a and C5a may be evaluated in the mouse models for clarification of their contributions to the "disease" state.

The incidence of mortality (40%) in irradiated-wounded mice with bacterial infections suggests that lesions exist at two portals of bacterial penetration: the skin-wound site or area and the intestinal tract. At the wound site, derangements in chemotaxis and phagocytosis may develop, which allow the colonization of bacteria that lead to death from sepsis. Chemotaxis and phagocytosis of peritoneal mononuclear cells may be used to evaluate these host defenses in single-injury and combined-injury hosts. Also, uncontrolled translocation of bacteria from the gut lumen into host tissues may occur. The result of massive translocation of intestinal bacteria in an immunocompromised host is septic death. The occurrence of translocation will be evaluated and quantified. Selective decontamination will be studied using antimicrobials to prevent sepsis. Diamine oxidase will be evaluated as a measure of the viability and ability of the mature intestinal cell to act as a physical barrier against bacterial invasion. A collaborative study to evaluate levels of intestinal mucin in single-injury and combined-injury hosts may explain if this physical barrier to bacterial penetration is altered in those kinds of injury.

Physiologic disturbances produced by single or combined injury will be monitored by clinical chemistry evaluations. The goals in this study are to determine the extent of damage induced after each injury situation, to identify the organ systems that are significantly injured after each single and combined injury, and to find clues explaining the mortality differences between irradiated-wounded and irradiated-burned hosts.

The main goals of the treatment modalities will be to prevent and cure infections, to provide support therapy, and to repair the injured lymphomyelopoietic system. Recommendations for research related to treatment are as follows:

Prostaglandin E_2 inhibitors such as indomethacin and aspirin will be used to prevent the burst of PGE_2 seen after single and combined injuries.

Since opsonic factors, especially IgG, are severely reduced after single and combined injuries, mice will be given homologous IgG infusions to determine the value of such therapy in terms of survival. The work will be done in irradiated-wounded mice with nosocomial or intentional infections.

Antimicrobial therapy with standard and microencapsulated antibiotics will be performed in sepsis, trauma, and combined-injury models.

Immunoenhancing compounds (such as glucan P, glucan F, and thymosin) and macrophage enhancers (such as pentoxiphylline) will be used in models of radiation, sepsis, trauma, and combined injury.

Passive immunotherapy with hyperimmune serum against specific bacteria-conditioned pathogens will be used in all injury models.

Investigations of combined treatment or therapy modalities will be based on the best results obtained for single treatment or therapy use.

IMMUNE AND HEMOPOIETIC ENHANCEMENT

PROBLEM

The hemopoietic syndrome is characterized by the destruction of a critical amount of hemopoietic and functional lymphoid cells, which results in suppression of the host's immunologic defenses and finally death from infection within 30 days. Destruction depends on the species, radiation dose, and radiation quality. Survival after exposure to radiation doses that induce hemopoietic and immune dysfunction depends on the therapy used. The AFRRI research program has emphasized two main areas of possible therapy, stem cell physiology and hemopoietic enhancement through biological response modifiers.

Stem Cell Physiology

Mechanisms are investigated that regulate stem cell self-renewal and differentiation into functional cells of the host defense system. Manipulation of the stem cell population will depend on knowledge of these self-renewal mechanisms and those that determine the balance between renewal and differentiation.

Stem Cell Isolation and Concentration

Current methodology centers on the use of physical and immunologic properties of the putative stem cell and associated cells of the hemopoietic system. The use of specific monoclonal antibodies associated with more sensitive fluorescent molecules has allowed more efficient use of the fluorescence-activated cell sorter (FACS), to isolate enough candidate stem cells for in vitro and in vivo analyses. Methodology that has already proved useful in the large-animal situation is the physical technique of counterflow centrifugation-elutriation. This has the advantage of rapidly separating large numbers of bone marrow stem cells from the immunocompetent lymphoid cells that are responsible for graft rejection. Further purification of the stem cell or associated cell populations can then be attempted, through use of a FACS or by other immunological techniques such as "panning" and/or complement-mediated lysis in combination with monoclonal antibodies.

In Vitro Culture Techniques

The ability to study the hemopoietic stem cell depends on methodology for culturing these cells in an in vitro system. This will allow us to detect and quantify the cells and manipulate their growth and differentiation in the culture dish. This methodology is being pursued in murine, canine, and primate models. Identification of the candidate stem cell in the canine and primate models requires the transplanting of autologous bone marrow.

Hemopoietic Enhancement

The regulation of an integrated host response to infection is imperative. In our concept, the host's response is made up of the stem cell population, the cells of the nonspecific inflammatory response, and the components of the cell-mediated immune response.

The primary approach used is the testing of potentially useful immodulators and/or biological response modifiers for their ability to (a) enhance hemopoiesis in normal and irradiated mice and (b) enhance the nonspecific inflammatory response and cell-mediated immunity in murine models of immune suppression and Gram-negative sepsis. A modulator with great potential is the polyglycan glucan F. Several other modulators are being investigated in collaboration with the Biological Response Modifiers Program of NCI.

PROGRESS

Hemopoietic Stem Cell Isolation (McCarthy)

The purpose of this study is to isolate by phenotypic characterization the colony-forming unit-spleen (CFU-s), a multipotent stem cell of the rat bone marrow. Using a specific panel of phycoerythrin and fluoroscein-labeled monoclonal antibodies, the CFU-s was isolated via pattern recognition on a FACS. The project required developing a method by which phycoerythrin could be coupled to the F' AB fragment of an immunoglobulin molecule. This method has reduced by a factor of 4 the time required to sort a quantity of CFU-s to be used in certain experimental procedures. Also, this procedure improved the resolution of the populations that are labeled with the dual fluorochromes. The result has been a 100-fold to a 150-fold enrichment of the CFU-s. The isolated CFU-s represented less than 0.5% of the original bone marrow population. This phenotypic characterization allows the detection of CFU-s that survive after exposure to ionizing radiation, thus enabling the bone marrow status to be assessed immediately. The CFU-s purification enables in vitro studies to be performed, to address questions on regulatory mechanisms.

Pluripotent Stem Cell Physiology: Large-Animal Models (Monroy, Vigneulle, Schwartz, MacVittie)

The objective is to evaluate the bone marrow syndrome in the dog after exposure in a high-neutron field, and to assess the effectiveness of neutron irradiation. In a dog model, postirradiation therapy regimens [fluids, antibiotics, platelets, and bone marrow transplantation (BMT)] and in vitro culture techniques were used to assay progenitor stem cells of the bone marrow and to improve bone marrow dosimetry. The results of the study are as follows:

The LD50/30 dose was determined at 1.48 Gy, which is $\geq$0.6 Gy less than previously published doses.

The neutron effectiveness was determined to be 1.5, compared to the X ray.

A correlation was established between progenitor cell kill and recovery with lethality.

The dose delivered to the bone marrow was established.

An LD50/30 was established for a unilateral (nonuniform) exposure in which damage to the GI tract complicates bone marrow failure.

It was concluded that, at >90% lethal dose, autologous BMT was only 60% effective in rescuing the dog.

Mismatched allogeneic BMT was not effective at all, with animals dying of graft failure. Matched allogeneic BMT was 60% effective in ensuring their survival.

Isolation of Bone Marrow Cell Populations Enriched for the Stem Cell (Monroy, Schwartz, Patchen, D'Alesandro, MacVittie)

The aim of this study is to rapidly isolate a stem cell population from bone marrow that is enriched for stem cell activity. The physical separation technique, counterflow centrifugation-elutriation (CCE), and bone marrow aspirated from dogs and monkeys were used. Stem cell activity was assessed with in vitro culture techniques and other parameters such as sizing, morphology, and antigenic expression of isolated cell populations. In a collaborative study with investigators at Johns Hopkins University, the isolated cell population is being purified using the human monoclonal antibody HLA-DR. Results are as follows:

A stem cell population was isolated from bone marrow of the rhesus monkey using CCE that has a low lymphocyte contribution and high in vitro progenitor cell activity, and is able to restore the bone marrow and rescue a lethally irradiated monkey.

The isolated cell population was further purified using the antigenic technique, E-rosette formation of lymphocytes with sheep red blood cells. This purified population retained the capability of rescuing lethally irradiated monkeys in the autologous and the allogeneic situations.

Panning and complement-mediated lysis techniques have been used to identify the antigenic expression of stem cells of the rhesus bone marrow. In vitro and in vivo assays have been performed.

Regulation of Hemopoietic Stem Cell Differentiation and Self-Renewal (Schwartz, Monroy, Neta, MacVittie)

This study was undertaken to understand the mechanisms that regulate stem cell self-renewal and the differentiation process in normal animals. Another objective was to develop procedures to manipulate the kinetics and differentiation of the pluripotent stem cell, to enhance the recovery of stem cells that survive exposure to ionizing radiation. The approach used the available stem-cell enrichment techniques: soybean agglutination (monkeys), CCE (mice and monkeys), and monoclonal antibodies, coupled with in vitro assays of primitive multipotent stem cells, CFU-GEMM, and CFU-s, which are now in use. We are evaluating the CFU-GEMM and CFU-s as predicators of pluripotent stem cell concentration in the bone marrow of the mouse and monkey models. In addition, the in vitro culture systems are evaluating specific growth factors for their role in causing the cell population to differentiate and/or self-renew. The growth factors will also be available from rhesus monkey and/or human T-cell hybridoma cell cultures, supplied under contract with the Oklahoma Medical Research Foundation. The growth factor thymosin is being evaluated for its enhancement of bone marrow recovery

postirradiation. The current data tend to support this product as an effective growth factor. A short-term assay (blast cell assay) is being developed, in which the capability of a primitive stem cell (the "S" cell) to self-renew is being evaluated. Collaborative efforts with investigators at NCI are using retroviral vectors to get at the question of genetic regulation of stem cells.

Hemopoietic Enhancement With Immunomodulators (Patchen, Neta, Brook, Ledney, Gruber, MacVittie)

The goal is to identify immunomodulators that have the capability to stimulate hemopoiesis in normal animals, and to apply those immunomodulators to models of irradiation, infection, or combined injury to evaluate their potential in enhancing the survival of those animal models. The mouse model was used to evaluate the polyglycans glucan P (particulate form) and glucan F (soluble form), for their capability to enhance survival after exposure to lethal doses of ionizing radiation. (The use of the glucans is in collaboration with Dr. N. Diluzio of Tulane University and Dr. P. Jacques in Brussels.) The glucan P and glucan F forms were shown to enhance the survival of mice exposed to radiation doses of 9-11 Gy. This enhancement was observed when the glucan was injected before exposure. In this mode, the glucan P form was more effective than the glucan F. However, when the glucan was administered after the radiation exposure, glucan P did not enhance survival, whereas glucan F did enhance survival. The survival was not as great as when the glucan was administered before irradiation.

Another objective of the study is to evaluate the effect of glucan P and glucan F on the macrophage. The release of various agents (inflammatory mediators, ectoenzymes, IL1, CSA, and others) were evaluated by isolated macrophages after exposure to glucan P or F. Inflammatory mediators released after exposure to glucan in vitro were identified. The induction of phagocytosis and microbicidal activity after glucan administration was observed. Ongoing studies include evaluating the following: ectoenzymes that are macrophage-specific; intracellular glutathione levels; expression of surface antigens; and the release of lymphokines, hemopoietic factors (IL2 and IL3), and other agents. Additional biological response modifiers are continually being evaluated in collaboration with Dr. Chirigos of NCI (Frederick) for hemopoietic enhancement.

RECOMMENDATIONS

The research program on hemopoietic dysfunction has progressed significantly. It is suspected that the antigenic markers found on rat stem cells are also expressed on higher animals (e.g., dog and monkey) and that the same approach for the purification of rat CFU-s can be applied to these animals as well. This study will be extended, with emphasis on developing a large-scale purification system with various affinity binding techniques.

Results of the research on physiology of pluripotent stem cells in large-animal models warrant extending the system to pulse mode, nonuniform exposures with shielding, and lower mixed-neutron levels.

The studies on stem cell isolation have already proved useful. The technique of counterflow centrifugation-elutriation has allowed rapid isolation of large numbers

of bone marrow cells containing the stem cell population. Research must continue to further purify the stem cell population for the physiology-regulating studies. Bone marrow transplantation will be used as the bioassay system for the pluripotency of the stem cell.

Investigation of stem cell self-renewal and differentiation must be continued in order to understand the mechanisms that regulate the process. It is planned that future research will use the purified stem cell populations in the monkey and mouse systems to assess the regulating requirements (cellular and growth factor) of the multipotent stem cells in undergoing either self-renewal or differentiation.

The use of glucans to enhance survival in mice after exposure to lethal doses of ionizing radiation has produced results that require further studies with the dog model. Glucans are also being applied in the combined-injury program. The potential of glucans for enhancing survival does support continued research.

PERFORMANCE DECREMENT

As a consequence of radiation exposure, profound behavioral and performance changes can be observed in both animal and man. These alterations vary with the type and the dose of radiation. The changes in the behavioral patterns are a consequence of either direct or indirect physiological changes in nervous system patterns and responses.

EARLY TRANSIENT INCAPACITATION AND PERFORMANCE DECREMENT

Problem

Precisely defining the occurrence of early transient incapacitation (ETI) and performance decrement after exposure to ionizing radiation is important for understanding the mechanisms responsible for the phenomenon and for developing mitigation techniques. Degradation of performance after irradiation can occur from any of a number of direct or indirect effects on the nervous system. It is known that the effects of radiation are dependent on the quality of the exposure, yet the occurrence of ETI, performance decrement, and emesis have not been fully defined for different qualities of radiation exposure. Recent research has sought to define radiation-induced performance decrement and to provide for the reduction of this response by

defining the effects of various qualities of radiation on motor performance,

characterizing the differences in the occurrence of emesis after neutron and gamma exposures,

investigating the etiology of radiation sickness,

evaluating the effects of radioprotectant compounds on performance, and

precisely modeling the ETI.

Progress

Different qualities of ionizing radiation produce different degrees of biological damage. Neutrons are consistently found to be more effective in killing organisms than are equivalent doses of gamma photons. On the other hand, gamma radiation has been shown to be more effective in producing ETI in monkeys (Young) and miniature pigs (George). In recent work designed to compare these findings with those for electrons, the motor performance of rats was used as a test model to determine the relative effectiveness of bremsstrahlung, electrons, gamma photons, and fission spectrum neutrons in producing performance decrement (Bogo). Again, neutrons were found to be the least effective. While there was no significant difference in the effectiveness of the two energies of photons (bremsstrahlung and cobalt-60), the bremsstrahlung tended to be more effective. Electrons were found to produce significantly more performance decrement than did the other qualities of radiation. These results imply that electrons are the most significant of these types of radiation in producing behavioral incapacitation because the response to the radiation varies directly as a function of the electron density generated by the respective qualities of radiation.

While experimental work has addressed the relative effectiveness of neutrons and photons in producing death and behavioral incapacitation, the relative toxicity of these radiations in causing emesis has not been defined. Recent work has addressed this problem by comparing the incidence and frequency of emetic episodes in monkeys after exposure to radiation fields of different qualities and energies (Young). Both high-energy and fission spectrum neutrons were found to produce significantly more frequent and longer lasting emesis than did comparable energy photons.

The mechanism for radiation-induced emesis is not fully understood. Recent work has sought to test the hypothesis that the emetic response to ionizing radiation is caused by the release of a humoral factor from injured cells, which acts on the area postrema (AP) in a manner similar to other toxic environmental stimuli. Using conditioned taste aversion (CTA) in the rat as a model for radiation sickness, CTA was produced by either two subthreshold doses of radiation or one subthreshold dose of radiation in combination with one subthreshold dose of a CTA-inducing chemical (lithium chloride) (Rabin, Hunt). These results are consistent with the idea that radiation sickness is induced via a humoral agent acting on the AP.

In addition to the work on the occurrence and mechanism of radiation effects on behavior, a series of recent experiments has addressed the behavioral toxicity of the proposed radiation-protectant material WR-2721 (Bogo). While this material has been shown to afford some protection against radiation lethality, it did not protect against radiation-induced performance decrement. Further, the drug itself significantly disrupted the ability of nonirradiated rats and monkeys to perform behavioral tasks. These studies clearly show that WR-2721 is a toxic substance in its own right and that it offers no behavioral protection against radiation toxicity.

Military requirements for information on radiation effects include research to characterize the behavioral phenomenon, elucidate the underlying mechanism, devise treatments, and develop precise quantitative models for planning. To meet this last need, a recently completed effort has provided a high-fidelity mathematical algorithm of ETI for use in Monte Carlo combat-simulation models. In addition to meeting a direct operational military need, this work clearly demonstrates, in a statistically significant manner, that neutron-rich radiation fields are less debilitating to behavior than are predominantly photon fields.

Recommendations

Research to define the mechanisms of radiation sickness and ETI will be continued and emphasized. This work is essential to successfully devising strategies for treatment and prevention. Behavioral responses to radiation, especially in the first hours after exposure, are demonstrably different from the later responses involving the other organ systems. Thus, the mechanisms underlying the behavioral response cannot be assumed to be the same as those mediating other forms of radiation injury. As such, the mechanisms of performance decrement will be given special research attention. The role of radiation quality in causing behavioral decrement is especially important, and it will be vigorously investigated because of the clear indications that neutron effectiveness in causing behavioral effects is quite different from neutron effectiveness in causing death. This is an important issue in military planning and the development of treatments. Again, this highlights the uniqueness of the behavioral response and emphasizes the need for focused work in the area of radiation effects.

NEURONAL MECHANISMS

Problem

The mechanisms underlying the behavioral changes as a direct or indirect effect of radiation exposure are unclear. These changes can lead to profound alterations in nervous system excitability, resulting in fatigue, weakness, nausea, vomiting, increased epileptiform activity, and altered electrical activity in the brain. The mechanisms for the effects of radiation on the nervous system are uncertain. Radiation may directly alter neuronal properties, such as ionic conductances, membrane composition, or cellular metabolism, perhaps through the generation of free radicals. Radiation could affect neural activity indirectly by changing the neuronal environment. This might result from such actions as a decreased blood-brain barrier, changes in brain blood flow, or altered glial function.

Progress

Approaches used to define radiation effects were hippocampal brain slice, neuromuscular junction, and anatomical correlates of both radiation damage and radiation effects on recovery from injury.

Hippocampal brain slice can be used as a model for altered activity of the central nervous system. The hippocampus is an area of the brain that is involved with memory and learning; it is particularly radiosensitive. Ongoing studies involve the following:

Effects of humoral agents that might increase in neural tissue following radiation (e.g., histamine, prostaglandins, neurotensin). Histamine increases cellular excitability in the hippocampus by blocking the calcium-mediated potassium current. (Pellmar)

Effects of free radicals and oxidizing agents that may mediate the actions of radiation. Peroxide with iron is now being used to generate hydroxyl free radicals in vitro. Results indicate a decrease in synaptic efficacy and impairment of action potential generation. Other oxidizing agents in use are N-ethylmaleimide and chloramine-T. Reducing agents and free-radical scavengers such as dithiothreitol (DTT) and glutatione are being tested to see if they counter the effects of free radicals and oxidizing agents. DTT tends to increase synaptic excitability. (Pellmar, Tolliver)

Direct radiation effects in vitro. Hippocampus will be irradiated in vivo and in vitro to distinguish direct effects from indirect effects. Studied in vitro, humoral effects will be eliminated. Due to large variability of the response in normal tissue, a very large number of observations will be required in order to obtain significant results with this approach. (Tolliver, Pellmar)

The mouse neuromuscular junction provides a mammalian model for synaptic transmission and a preparation for the study of radiation effects on the peripheral nervous system. Radiation of tissue in vitro caused a dose-dependent biphasic

change in the frequency of spontaneous transmitter release, and similarly changed the amplitude of evoked release. The amplitude of unitary spontaneous events was unchanged. These results can be explained by an effect of radiation on the presynaptic mechanisms without alteration of postsynaptic receptors or transmitter-inactivation mechanisms. The range of radiation doses will be extended in future studies. The results from these in vitro exposures will be compared to similar studies using in vivo irradiation. (Livengood)

The anatomical response of avian brain to radiation provides structural correlates of radiation damage. The system also allows study of the effects of radiation on recovery from a conventional injury. The changes that occur in the avian brain with a localized surgically-induced injury have been analyzed. Radiation damage will be evaluated by a number of characteristics: number of degenerating presynaptic profiles, area occupied by postsynaptic dendrites, presence of mast cells, numbers of macrophages and erythrocytes, astrocyte proliferation, and capillary and blood-brain barrier integrity. Other techniques such as dye marking of single cells and labeling of transmitters in vesicles may be applied in future studies. (Phillips)

Recommendations

The physiology of the nervous system is more sensitive to ionizing radiation than previously thought. The mechanism of this sensitivity is unclear. Current evidence indicates that it may involve the effect of free-radical production on the ability of neurons to function normally. In this it may be closely related to the pathophysiology of the nervous system found with ischemic insult. These studies will be extended over the next several years because they appear to give insight to nervous system damage from a large number of potential insults, of which radiation is an important subset.

BRAIN MECHANISMS

Problem

Determination of the mechanisms underlying decrements in performance as induced by ionizing radiation is an important goal of the AFRRI mission. A number of approaches have been taken over the years in pursuit of this goal. These approaches have explored several broad areas of research, including studies of blood pressure, cerebral blood flow, blood-borne factors, neural excitation, and synaptic transmission. The results obtained have depended largely on the dose and quality of radiation and, indeed, the behavioral alterations found under the experimental conditions used.

The behavioral effects of ionizing radiation fall into three general categories. Generally, doses of 0.2-10 Gy are associated with emesis, conditioned taste aversions, and increased susceptibility to seizures. After doses of 10-15 Gy, behavioral activation has been reported in some rodents. Higher doses have a significant depressant effect on behavior that has been described as general performance decrement, ETI, or permanent incapacitation. The sensitivity of animals to performance decrement depends on dose and quality of radiation, the species used, and the behavioral task studied.

Progress

The physiological basis of the behavioral effects of radiation has been investigated for some time. For example, exposure to ionizing radiation can induce an early transient hypotension. This hypotension has been hypothesized to lead to a reduction in cerebral blood flow, which in turn could be a possible basis for ETI. Early studies measuring blood flow in whole brain had not demonstrated any clear changes after irradiation. However, in more recent experiments using 100 Gy gamma radiation, regional blood flow was reduced, especially in the hippocampus, an area particularly sensitive to anoxia-ischemia (Cockerham). In these studies the reduction of hippocampal blood flow followed a time course similar to ETI. Blood pressure was also reduced. Plasma concentrations of histamine (released from mast cells) and neurotensin (a stimulant of histamine release) were elevated after irradiation. Pretreatment with disodium chromoglycate (a mast cell stabilizer) blocked the radiation-induced reduction in hippocampal blood flow but not blood pressure. Other investigators have shown that disodium chromoglycate blocks the neurotensin-stimulated release of histamine from mast cells. These results suggest that neurotensin-sensitive compartments of histamine in the brain underlie these effects.

Information has accumulated to suggest that, after irradiation, the blood-brain barrier opens to substances that normally do not pass into the brain (Catravas). Since the blood-brain barrier is composed in part of endothelial cells, experiments have been initiated to determine whether exposure to ionizing radiation can directly damage these cells, particularly as pertaining to vascular permeability (Cerveny). Using cultures of endothelial cells as a model, various end points that address the morphological, functional, and replicative properties of these cells are being developed (cell count, $_3$H-thymidine uptake, protein content, lactate dehydrogenase activity, low-density lipoprotein incorporation, VWF immuno-fluorescence, clotting Factor V content, fibronectin ELISA, and 6-keto-prostaglandin F1-alpha). Preliminary experiments indicate that exposure to ionizing radiation (2.5 and 20 Gy) induces morphological changes, suggesting cell swelling and death.

Behavioral experiments have indicated that exposure to ionizing radiation degrades motor performance (Bogo). The proper coordination of movements depends on the normal function of several areas of the brain, including the basal ganglia. Examination of the role of dopamine (an important inhibitory transmitter in the caudate nucleus of the basal ganglia) has revealed alterations that seem to correlate with some of the behavioral changes previously reported (Hunt). An approach was developed that assesses the activity of dopaminergic neurons by measuring the steady-state concentration of the metabolites of dopamine. Increases in the concentrations of metabolites reflect enhanced electrical activity of nigrostriatal fibers, whereas decreases in the concentrations reflect a reduction in electrical activity. Using this technique, dose-response studies have demonstrated that at doses of radiation at which behavior was stimulated (15 Gy), dopaminergic transmission was enhanced. On the other hand, at higher doses of radiation (100 Gy) at which behavior was depressed, dopaminergic transmission was suppressed.

An understanding of how radiation might interfere with the receptors on which neurotransmitters act is important in assessing the role of alterations in synaptic transmission in radiation-induced performance decrement. Previous studies have reported increased cholinergic activity in the brain and heart after irradiation. Subsequent experiments at AFRRI were undertaken to determine the relationship between altered cholinergic activity and muscarinic cholinergic receptors (Durakovic). The receptors were examined by measuring the binding of quinicyclidinic I-125 4-iodobenzilate (4-QNB) after its injection into rats exposed to 6 Gy of gamma or neutron radiation. No changes were observed in the cerebral hemispheres, cerebellum, or pituitary gland. However, binding of 4-QNB was significantly reduced in the midbrain.

Other experiments have suggested a role for opioid peptides in the stimulatory behavioral effects of radiation (Mickley). Exposure of C57BL/6J mice to ionizing radiation in doses of 15 Gy results in locomotor hyperactivity, an action similar to that found after injections of morphine. Subsequent studies have been undertaken to localize the areas of the brain that mediate both these effects. In a study using the rate of uptake of C-14-2-deoxyglucose, intracerebral injections of the opiate antagonist naloxone, and lesions to different areas of the brain, results showed that the posterior nucleus accumbens, dorsomedial caudate nucleus, and lateral septum mediate the effects of morphine on locomotion. Radiation-induced hyperactivity does not appear to involve the nucleus accumbens. However, lesions of the other two areas of the brain studied and intracranial injections of naloxone into those areas attenuated the stimulatory effect of radiation.

In an effort to gain insight into the possible underlying mechanisms of changes in synaptic transmission, experiments were undertaken to evaluate the ability of neurons to initiate appropriate electrical signals for allowing information to be properly transmitted through the brain and to the periphery. We used as a model the ability of synaptosomes (isolated nerve endings) to take up ^{22}Na after stimulation by neurotoxins that open sodium channels. We found that exposure to ionizing radiation in doses of 1-1000 Gy significantly reduced the initial rates of sodium uptake in a dose-dependent manner (Mullin). Since membrane lipids are important in the function of the sodium channels, measurements of the steady-state fluorescence of molecular probes in synaptic plasma membranes were performed. The measurements revealed no changes in the fluidity of membranes exposed to 100 and 1000 Gy of radiation. This observation suggests no apparent structural (confirmational) change in the membrane lipids that could account for the effect of radiation in sodium uptake.

Several new approaches to study the neural mechanisms of performance decrement have been initiated that may prove valuable. One is the transplantation of brain tissue (Mickley). With this technique, embryonic brain tissue is transplanted into areas of an adult brain that have been previously lesioned. The transplants grow and in effect replace the damaged tissue. In addition, lesion-induced deficits in brain function can be reversed. Now established at AFRRI, this technique is being applied in an attempt to restore function to areas of the brain lesioned with ionizing radiation.

Quantitative autoradiography is being established to study the effects of radiation on cerebral metabolism and neuroreceptors (Movius). The technique involves injecting C-14-2-deoxyglucose or other radioligands and then determining the amount of ligand in different areas of brain slices. The intensity of labeling of the isotope is measured using a computerized imaging system. Methodology has been developed to allow investigations of rat brains.

Finally, investigations have been initiated to determine whether animals can be overtrained to the point where disruption of performance can be reduced by exposure to radiation (Harris) and whether animals can be conditioned behaviorally to stimulate their immune system (Mickley). These studies are in the formative stage.

Recommendations

It is clear from the presentations in this session of the program review that exposure to ionizing radiation results in a myriad of effects on the brain and on other systems that may have consequences on brain function. Although a relationship among the various observations is not yet clear, there are enough similarities to warrant the following recommendations.

Valid and relevant comparisons of neurobiological or other physiological mechanisms to changes in behavior cannot be made without a well-described profile of the behavioral effects of radiation. Further studies will be initiated that will provide a better understanding of these effects, especially at lower, sublethal doses.

The question of a role of histamine in the behavioral effects of radiation is not resolved. Many interesting and promising observations (including those described earlier on cerebral blood flow) have been made by several laboratories at AFRRI. A coordinated multidisciplinary approach will be initiated to determine conclusively the importance of radiation-induced histamine release to performance decrement.

The apparent opening of the blood-brain barrier after irradiation may have profound significance, considering the many changes in blood chemistry observed after irradiation. Substances that normally do not cross the barrier may do so after irradiation, having adverse physiological consequences. This possibility will be tested. Further studies will be conducted on irradiated cultured endothelial cells to further characterize the various cellular responses, including experiments with cultured brain capillaries. The effects of histamine, neurotensin, and putative radioprotectants will be examined.

Normal behavior depends on the integrity of the mechanisms involved in the generation of action potentials and synaptic transmission in the brain. Future investigations will include further characterizations of the effects of radiation exposure on sodium and calcium fluxes, neurotransmitter release, and neuroreceptors, and also examine possible transmitter interactions. Any observed changes need to be localized to specific brain areas and then associated with specific behavioral effects of radiation. A role for the actions of free radicals will be investigated.

Since radiation exposure can have many effects on the brain, with each effect possibly related to a particular behavioral decrement, pharmacological agents that alter brain function will be tested as potential radioprotectants against performance decrement.

New approaches to the study of radiation-induced performance decrement will be encouraged. The complexity of the problem requires imaginative ideas. State-of-the-art biological procedures will be developed where any reasonable probability of success is possible. The latest development in imaging techniques along with sophisticated behavioral and neurobiological approaches will be pursued and supported.

RADIOPROTECTORS

PROBLEM

The U.S. military services have established objectives for dealing with the problem of radiation and the use of chemical radioprotectants in a military environment. The three main objectives are to maintain military performance, increase survival, and produce a psychological advantage. The current goal is to field a drug (most likely WR-2721, S-2-[3-aminopropylamino] ethylphosphorothioic acid) by 1988. The desired qualities in a chemical radioprotectant are that it provide a dose reduction factor (DRF) of at least 2, be self-administrable (preferably oral), afford protection for at least 4 hours, have minimal side effects, be stable for 2-5 years, be compatible with other drugs, and not be abusable. Clinical studies of patients receiving WR-2721 have shown that this drug has toxicity problems that must be adressed, e.g., hypotension, diarrhea, nausea, vomiting, and changes in calcium metabolism. Animal studies at AFRRI have also indicated toxic effects of WR-2721 that would compromise its use in a military setting, notably a performance decrement. Some of the basic questions concerning the development of radioprotective measures superior to that now available will be addressed in this review.

Radioprotection is generally considered to refer to the administration of chemical compounds shortly before radiation exposure in order to increase postirradiation survival. In our studies at AFRRI, we have broadened our concern to include various points of intervention in relation to the time of radiation exposure, and consideration of potential treatment modalities for combined injury. Potential treatments for radiation injury take into consideration the various proposed mechanisms of radioprotection at the molecular level (oxygen radical scavenging, hydrogen transfer reactions, mixed disulfide hypothesis) and at the biochemical-physiological level (induction of hypothermia, hypoxia, biochemical shock; modification of cellular metabolism and enzyme induction; stimulation of immune responses). Several studies at AFRRI are concentrating on the importance of endogenous protective systems in relation to cell radiosensitivity and mechanisms of radioprotection (glutathione and enzymes that scavenge reactive oxygen species and metabolites). It is probable that no single hypothesis or method of radioprotection will explain completely radioprotection and repair. A combination of more than one agent may be necessary to attain the highest level of protection.

PROGRESS

Comparison of Sulfhydryl Compounds

Survival: Metabolism and Toxicity (Weiss, Jacobs, Kumar)

To arrive at a radioprotective regimen superior to that now available, it is necessary to obtain biological information about potential radioprotective agents. It is of interest to determine whether various sulfhydryl compounds have an advantage over WR-2721 in terms of protection, toxicity, route of administration, and eventually in potential use in drug combinations. Diethyldithiocarbamate (DDC) has been compared to WR-2721 because DDC is an immunomodulator of low toxicity under intensive clinical and experimental investigation. The effect of WR-2721 and DDC on the postirradiation survival of mice was determined, using

equitoxic doses of the drugs at one-half the maximum tolerated dose or one-half the LD10 dose. This is 400 mg/kg for WR-2721 and 800 mg/kg for DDC. When mice were irradiated with cobalt-60 at 100 rad/min, the DRF for WR-2721 was approximately 3, whereas for DDC it was 1.3.

Studies on the toxicity of radioprotective agents are in progress. Specifically, these include the behavioral toxicity of WR-2721 in rats and monkeys (Bogo), changes in biochemical parameters in animals after WR-2721 administration, and changes in calcium metabolism in mice treated with radioprotectants. Mass spectrometric analysis of metabolites of WR-2721 and DDC in cell preparations has been initiated.

Immunoprotection (Weiss, Jacobs)

The data shown above suggested that sulfhydryl immunomodulators can also have radioprotective activity, and it was of interest to determine the relationship between immunomodulatory and radioprotective effects. A model was developed for studying the effects of drugs on radiation-induced immunosuppression. Delayed-type hypersensitivity (DTH) provides the best information on overall immune status, even though DTH represents a complex interaction of lymphocyte, macrophage, and lymphokine activities. Mice were sensitized with oxazolone before irradiation and then challenged after irradiation. The greatest effect was observed when drugs were administered one-half hour before irradiation, as with survival studies. Protection of cellular immunity as measured by the DTH response was greatest for WR-2721 > 2-mercaptoethylamine > N-acetyl cysteine > AET > glutathione > DDC. No correlation was seen between the obtained protection and the concentration of injected sulfhydryl moiety. Although the specific mechanism of protection (such as protection of lymphocytes and/or macrophages) is not known, this procedure appears to be useful in the assessment of drugs for protection against radiation-induced decrements in cellular immunity.

To test the efficacy of drugs administered orally (p.o.), we compared the radioprotective effects of WR-3689 to WR-2721. WR-3689 has one additional methyl group on the amino group, and according to some previous work, it may be more effective than WR-2721 when given orally, mainly because it is less toxic. Drugs were administered at one-half the maximum tolerated dose: i.p. WR-2721 400 mg/kg, i.p. WR-3689 450 mg/kg, p.o. WR-2721 700 mg/kg, and p.o. WR-3689 1000 mg/kg. Given i.p., both drugs protected cellular immunity to the same degree, but p.o. administration of WR-2721 was slightly more effective when given 1 hour before irradiation. Similarly, WR-3689 did not provide an advantage in protecting against death when given either i.p. or p.o.

Protection against Neutrons (Jacobs, Steel)

Since WR-2721 is not as effective a radioprotectant against neutron irradiation as it is against gamma irradiation, it is necessary to determine if any other drugs are more effective protectants against neutron damage. It was found that, compared to irradiated controls, WR-2721 afforded the best protection against gamma irradiation and WR-151327 provided the best protection against neutron irradiation.

Metabolism of Radioprotectants: In Vivo Dephosphorylation of WR-2721 Monitored by Phosphorus Nuclear Magnetic Spectroscopy (Knizner, Jacobs, Lyon, Swenberg)

WR-1065, the dephosphorylated form of WR-2721, is proposed to be the active radioprotective form of the drug in vivo. Information concerning the in vivo dephosphorylation kinetics of WR-2721 was not available previously. ^{31}P NMR is a noninvasive and nondestructive technique that can be used to follow the dephosphorylation of WR-2721 and also the simultaneous production of inorganic phosphate. Male CD2F1 mice were injected i.p. with 600 mg/kg WR-2721. The half-life of WR-2721 in these mice, determined by phosphorus nuclear magnetic spectroscopy, was 40.9 ± 5.9 min.

Mechanisms of Radioprotection: DNA (Giambarresi)

To determine factors that contribute to the mechanisms of radioprotectant drugs in vivo, the effects of WR-2721 on DNA synthesis was investigated. The regenerating liver of the rat was used as the primary in vivo model system. Male F344 rats were trained to an "8 + 16" feeding regimen. A two-thirds partial hepatectomy (PH) was performed at the end of the 12-hour dark cycle, and WR-2721 was injected 13 hours later. At several time points after PH, tritiated thymidine was injected i.v. One hour after injection, the liver and proximal 10 cm of duodenum were removed. DNA from the liver and gut was quantitated, and the incorporation of labeled thymidine into the DNA of these tissues was determined. Mitotic activity was determined on histologic preparations of liver tissue. Administration of WR-2721 was followed by a markedly decreased incorporation of tritiated thymidine into liver DNA at all time points. This inhibition of DNA synthesis was also reflected by a profound inhibition (80%) of mitotic activity in the livers of the WR-2721-treated rats. DNA synthesis in intestine was also inhibited. In both regenerating liver and gut, the inhibition of DNA synthesis was found to be related to the dose of WR-2721 administered. These findings are consistent with those for other sulfhydryl radioprotectants in a number of other model systems. Inhibition of cell proliferation appears to be a common effect of these agents, and inhibition of DNA synthesis may play a role in the radioprotective effects of these compounds in vivo.

Mechanisms of Radioprotection: Polyamines (Past, Giambarresi)

Polyamines are a class of compounds that are critically important for normal cell growth and differentiation. It is therefore not surprising that these compounds are present in high concentrations in actively dividing tissues. Polyamines have been shown to be essential in the recovery of tissues such as the intestinal epithelium from chemotherapy-induced injury. Recent work from the USSR has indicated that polyamines administered after radiation may increase the survival of animals and enhance GI recovery. The mechanisms of action through which polyamines protect tissues from radiation damage is not known. However, polyamines may bind directly to cellular DNA. In this new work unit, the role of polyamines in radiation damage and protection will be investigated. The progress to date involves development of methods for separating and quantifying polyamines.

Mechanisms of Radioprotection: Lipid Peroxidation (Weiss, Catravas, Kumar, Vaishnav)

The role of membrane lipid peroxidation in relation to radiation injury, protection, and sensitivity is under investigation. When peroxidation of unsaturated fatty acids in cell membrane phospholipids occurs, among the products formed are volatile hydrocarbons. When arachidonic acid, for example, is attacked by a free radical such as OH, and in the presence of oxygen, a hydroperoxide is formed that can decompose, and readily if ferrous ion is present. One of the decomposition products is pentane. By gas chromatography, the volatile hydrocarbons produced in model systems such as red cell membranes can be measured over periods of time. It was found that small amounts of pentane are evolved upon irradiation, but the presence of ferrous ion is needed in order to see a substantial increase. Pentane production could be suppressed when WR-2721, other radical scavengers (benzoate, superoxide dismutase, catalase), or the iron chelator desferrioxamine were present during irradiation. This model system and similar ones provide basic information on the chemical events occurring during radiation exposure, and provide ideas on potential radioprotective agents.

The ability to determine volatile hydrocarbons expired by irradiated animals is also under consideration. Individual rats are placed in a chamber in a closed system in which pure oxygen is introduced. The air is circulated through various traps, and air samples are collected and adsorbed on a cartridge that is put into the gas chromatograph for analysis. Rats irradiated with 1000 rads appear to expire increased levels of pentane, but this is especially clear when the animals are pretreated with iron dextran. Radiosensitizers that are thought to deplete glutathione were also given before irradiation, and the subjects expired increased pentane after irradiation. The studies indicate that increased radiation injury might occur in individuals when iron occurs in an unbound form.

Mechanisms of Radioprotection: Endogenous Protective Enzyme Systems (Kumar, Weiss, Blakely)

Glutathione peroxidase, superoxide dismutase, and catalase detoxify free radical species of oxygen that are produced from ionizing radiation or the products of their interaction with the cell milieu. Therefore, the modulation of their activities by radioprotectants may be of importance in radioprotection. In the present investigation, the role of WR-2721, DDC, and WR-3689 in modulating the activity of glutathione peroxidase was studied. WR-1065 (the sulfhydryl metabolite of WR-2721) and DDC inhibited the mouse-liver cytosol glutathione peroxidase in vitro, probably by competing with the natural substrate, reduced glutathione. When WR-2721 and DDC were given i.p. for short intervals of 1, 2, or 3 hours, the activity of the enzyme in liver was enhanced from 30% to 40% at the end of 2-3 hours. No effect was seen at the end of 1 hour, and the kidney enzyme was not affected. Drugs were incorporated into diets that were fed 7 days before irradiation. Tissue enzymes were assayed 7 days after irradiation. Glutathione peroxidase activity decreased by 30% in irradiation control animals, whereas enzyme activity in the liver was similar in nonirradiated animals and in irradiated mice that were fed diets containing WR-2721, WR-3689, or DDC. The enzyme in the kidney did not show similar patterns; higher levels were seen in the irradiated animals, and only DDC treatment resulted in increased enzyme activity. Other studies indicated that DDC, in contrast to other sulfhydryl protectants, may stimulate the hexose monophosphate shunt pathway.

In order to elucidate the role of glutathione peroxidase in cellular radioresistance, cell lines of known differing radiosensitivity were tested for their enzyme activity. That is, the enzyme activity was compared in human kidney T1 cells and the very radiosensitive ataxia telangiectasia (AT) cells (courtesy of E. Blakely). It was found that AT cells had only one third of the glutathione peroxidase activity of T1 cells. These studies indicate that glutathione peroxidase has an important function in determining the radioresponsiveness of cells, and it may be a factor in the mechanism of action of radioprotective agents.

Naturally Occurring Radioprotective Agents (Past, Jacobs)

Several organisms are capable of surviving high doses of ionizing radiation. Among the radioresistant organisms are some members of the Deinococcus family of bacteria, including D. radiodurans. It has been shown that an extract isolated from D. radiodurans confers radioprotection to a more radiosensitive strain of bacteria, E. coli. The isolation and characterization of this naturally occurring radioprotectant is in progress. A crude radioprotective fraction isolated from D. radiodurans exhibits a characteristic spectral absorbance at 207 nm and 263 nm. Amino acid analysis of the crude radioprotectant fraction has revealed the presence of various amino acids, including sulfhydryl-containing amino acids. Further purification of the crude radioprotective fraction has indicated the presence of two major components. Male CD2F1 mice were injected i.p. with the factor 60 minutes before irradiation with 1000 rads cobalt-60. Mice treated with 150 mg/kg showed increased postirradiation survival (10%) and protection against weight loss, compared to irradiated controls. Increased 30-day survival was also observed after treatment with a higher dose of the factor.

Nutrition: Combinations of Radioprotectants (Jacobs, Weiss)

To maximize survival after radiation exposure, various agents or procedures can be considered for administration at various time periods with respect to radiation exposure. Sulfhydryl antioxidants, nonsulfhydryl antioxidants, and dietary factors might be more effectively given before irradiation, whereas immunostimulants may be active on surviving cell fractions after irradiation takes place. (See also Immunologic/Hematologic Enhancement, Patchen and Neta.) Bone marrow transplants can be administered after radiation if no surviving cell fraction is present. Antibiotics can be administered later if infection develops. We have recently studied a number of potential radioprotectants other than sulfhydryl compounds and administered them either by i.p. injection, as constituents of drinking water, or in the diet. These included antioxidants, enzymes, immunomodulators, purine, oxathiazolidine, vitamins, and minerals. Some compounds have been given in combination with WR-2721, but nothing tested thus far has provided protection greater than that afforded by WR-2721 alone. Studies with nutritional factors have shown that either vitamin E (three times the normal diet) or selenium (4 ppm in drinking water) will increase the DRF for 30-day survival to 1.2. It was found that vitamin E protects against the loss of glutathione peroxidase activity, whereas selenium actually increases the enzyme activity about 35% above the level attained on the normal diet and drinking water. Combined use of vitamin E and selenium also protects against weight loss in irradiated mice.

Liposomes as Delivery Vehicles for Radioprotectants (Snyder, Walden, Patchen)

The aim of this research is to determine whether the therapeutic efficacy of WR-2721 or other radioprotective drugs can be enhanced by incorporating them in liposomes. Techniques for incorporating and quantifying radioprotectants in liposomes have been established. Three WR compounds were encapsulated in liposomes (WR-2721, WR-215, WR-2691) and tested for their radioprotective effect in mice. No significant enhancement was observed when these compounds were encapsulated and administered either i.v. or i.p. However, the quantity of the drug that can be delivered in liposomes is limited by their relatively small "capture volume" and the total amount of the lipid that can be administered practically. It was therefore decided to explore the possibility that certain "nonspecific immunoenhancing" agents active in microgram quantities could be potentiated as radioprotectants when delivered in liposomes. In fact, muramyl dipeptide (MDP) increased the survival of lethally irradiated (1000 rads cobalt-60) mice to 20%-30% when given i.p. or i.v. in liposomal form.

Endotoxic lipopolysaccharide (LPS) has long been known to be radioprotective. Nevertheless, the toxicity of LPS, or its principal bioactive component Lipid A, has detracted from its potential use as a radioprotectant. Recently a detoxified form of Lipid A became commercially available. We have conducted experiments showing that the detoxified form of Lipid A (monophosphate) retains the radioprotective properties of the native LPS or Lipid A (diphosphate). The radioprotective effect of Lipid A was enhanced when it was incorporated in fluid liposomes, whereas the effect was diminished when liposomes were more solid. Although both LPS and the toxic form of Lipid A (diphosphate) stimulated hematopoietic recovery in irradiated mice as measured by endogenous spleen colony formation (CFU-s), no such effect could be demonstrated using detoxified Lipid A. These observations are important for two reasons. First, they indicate that the radioprotective properties of LPS are not necessarily linked to the biologic activity of the molecule responsible for toxicity. Second, the mechanism of radioprotection by LPS or its derivatives may be independent of its ability to stimulate hematopoiesis.

RECOMMENDATIONS

The search for sulfhydryl and nonsulfhydryl radioprotectants superior to WR-2721 with respect to in vivo radioprotection and toxicity will be continued. Emphasis will be placed on effective oral radioprotectants or the possibility of using newer drug-delivery systems. Drug doses that are not behaviorally toxic in mice will be determined. Continued studies of in vivo immunoprotection by potential radioprotective agents will be correlated with determinations of protection of subpopulations of immune cells (e.g., lymphocytes). The effect of protectants on cell function (e.g., macrophage, thymus cells) and on circulating factors that affect immune responses (acute-phase proteins) will be studied. Studies will continue on (a) the toxic effects of sulfhydryl compounds on calcium metabolism and (b) the HPLC, gas chromatographic, and mass spectrometric analysis of metabolites of the most effective protectants, in order to elucidate the possible active factors functioning at the cellular level.

The dephosphorylation kinetics of phosphorothioate compounds other than WR-2721 will be determined by nuclear magnetic spectroscopy, and the in vivo metabolism of other drugs can be followed. Single-cell studies dealing with radioprotective drugs can be initiated.

Characterization of the inhibitory effect of WR-2721 on DNA synthesis will be continued by extending studies to 4 weeks and examining the time delay of peaks of DNA synthesis and mitosis, alterations in liver growth curve, time delay for complete regeneration, and optimum time to administer WR-2721 with respect to partial hepatectomy to obtain an indication of the cell cycle specificity of action of WR-2721. The effect of WR-2721 on DNA-metabolizing enzymes will be studied in order to understand how WR-2721 inhibits DNA synthesis and mitosis.

For the new study of polyamines in radiation injury and protection, it is proposed to examine the effect of gamma radiation on polyamine levels and polyamine biosynthetic enzymes in two rapidly dividing in vivo cell populations: the epithelium of the small intestine and regenerating rat liver. The effect on postirradiation survival of different polyamines will be determined. Owing to the similarity in structure of WR-2721 and polyamines, it is planned to examine the effect of the drug on polyamine levels and polyamine metabolic enzyme activities, and to determine if combined treatment with polyamines and WR-2721 will provide increased protection and reduced toxicity.

Volatile hydrocarbon production will be studied in vitro using other cell systems. The production of volatile hydrocarbons (ethylene, pentane) in cell-free and incubating cell systems can be used for elucidating the reactive oxygen species involved in the radiation effect under study. Information obtained on potential radioprotectants using the in vitro model systems will be applied to mouse survival studies. The technical procedures used for in vivo analysis of volatile hydrocarbons will be improved to maximize sensitivity. Other hydrocarbons produced by irradiated and drug-treated rats will be identified by GC-mass spectrometry in order to determine whether the other hydrocarbons are more relevant to injury than pentane. Other factors responsible for hydrocarbon evolution by rats will be determined.

We will continue and complete the present studies on the interrelationship of radiation, radioprotective drugs, and protective enzymes (glutathione peroxidase, superoxide dismutase, catalase) in various tissues (liver, kidney, spleen, bone marrow). Enzyme changes will be studied in cultured cells at different stages of the cell cycle and in cell lines of differing radiosensitivity, to determine the relative importance of the enzymes in endogenous radioprotection. The use of cell cultures, in addition to mice, will be examined to determine the role of enzyme induction in radioprotection by potential agents. If exogenous augmentation of enzyme levels (by injection into animals or in cell cultures) does not optimally elevate the intracellular levels of enzymes, then compounds simulating these enzymes and structural modifications of the sulfhydryl compounds may be explored in order to improve radioprotection.

The two major components of the radioprotectant fraction from D. radiodurans will be separated by preparative reverse-phase HPLC to obtain the two components in milligram quantities. The radioprotective and toxic properties of the two separated fractions will be determined in mice. The radioprotective fraction will be structurally characterized in order to gain knowledge useful in the design of effective and nontoxic radioprotectants.

Additional studies need to be done on the radioprotective effects of compounds with diverse mechanisms of action (e.g., nonsulfhydryl antioxidants, immunostimulants, metabolic inhibitors, chelating agents), administered alone or in combination with WR-2721. Of critical importance is information on potential protectants against GI and CNS death. Studies on protection against CNS toxicity will be performed in mice using an accelerod. Combinations will use a minimum amount of WR-2721 that will provide protection but low toxicity. Continued study is warranted on the effect of nutritional factors (especially vitamins E and A, selenium, zinc) on postirradiation survival, endogenous protective systems, and immune function. Studies on dietary factors in combinations will be aimed at the possibility of making dietary recommendations for active-duty military personnel with respect to radioprotection.

The structure of liposomes will be systematically altered in an attempt to enhance their utility as delivery vehicles for radioprotectants, because the structure of liposomes can drastically alter their pharmacologic distribution and kinetics. In addition, liposomes are ideal candidates for administering combination regimens because water-soluble drugs (WR-2721, glucan, etc.) can be trapped in their aqueous spaces, while lipophilic radioprotectants (Lipid A) can be incorporated directly in their membranes.

STATUS OF IN-HOUSE RESEARCH AT AFRRI

BEHAVIORAL SCIENCES DEPARTMENT

Group Title: Experimental Psychology Division

Objectives

To develop and validate animal models of human performance relevant to military missions.

To utilize animal models to characterize the effects of ionizing radiation on human performance in military situations.

To define and describe the magnitude, duration, and time course of radiogenic performance degradation and early transient incapacitation (ETI).

To develop and refine probabilistic models of performance degradation and ETI for use in computerized combat effectiveness models (e.g., AURA).

To evaluate the behavioral effects of radioprotectants.

To evaluate the effects of different radiation qualities and ratios of nuetron/gamma radiation on performance.

To evaluate the effects of combined injury on locomotor performance.

To evaluate the effects of radiation on social behaviors.

To determine how ionizing radiation alters strength and duration of behavioral responses.

To utilize brain grafting as a means through which mechanisms of radiogenic behavioral deficits may be deduced.

To determine the role of endogenous opiates and opiate receptors in the production of radiation-induced behavioral change.

To examine the effects of ionizing radiation on both simple and complex learning and performance in animal models.

To determine if radiation alters behavioral sensitivity to drug and chemical treatments.

To examine neurochemical mechanisms of radiation-induced behavioral change.

Current Status

e are determining effects of radiation quality on rodent motor performance.

.e are determining effects of radioprotectants and radiation on rodent motor erformance.

We are recording the strength and du·tion of the behavioral responses of rats.

We are evaluating the methods of radiation-induced hippocampal damage and recording correlated behavioral changes.

We are exploring the use of the rat in studies of endorphin's role in radiogenic behaviors.

We are evaluating the effects of combined radiation, burn, and wounding on locomotor performance.

We are defining the usefulness of appetitive reinforcement as a motivator of irradiated rats.

Future Goals and Directions

Obtain the ED50 for rats tested 3 minutes after exposure to electron, gamma, and neutron radiation.

Determine, through the use of modeling techniques, why electron radiation is more effective at producing performance decrement than other radiation qualities and why neutron radiation is the least effective.

Test candidate radioprotectants at the LD50 dose of gamma radiation to determine effects on rat motor performance and lethality.

Test other radioprotectants, such as vitamins or minerals and/or other WR compounds, to determine their efficacy in reducing radiogenic performance decrements.

Adapt the accelerod task to evaluate mouse motor performance after radiation exposure.

Test for the ability of antiemetics to protect from performance decrement caused by radiation exposure.

Test for the ability of head or partial body shielding to mitigate radiation effects.

Test for the effects of combined neutron/gamma radiation on performance.

Study the influence of radiation on social behaviors and the behavioral pharmacology of social behaviors.

Study involvement of CNS autostimulation of the immune system of irradiated mice.

Determine the role of delta opiate receptors and neurotension in the production of radiation-induced behaviors.

Determine the influence of practice and overtraining on postirradiation strength and duration of performance.

Measure the effects of ionizing radiation on both simple and complex learning and performance in animal models.

Accomplishments

It was concluded that the relative biological effectiveness (RBE) of high-energy electrons is greater than either bremsstrahlung, gamma, or neutron radiation for producing performance decrement of rat motor performance and that neutron radiation was the least effective of the four fields.

We found that, at therapeutic doses, the radioprotectant WR-2721 produces significant decrement in rat motor performance and monkey speed-stressed visual discrimination (SSVDT) performance. WR-2721 does not protect against radiation-induced performance decrement or ETI under these circumstances. These monkey data have been added to the primate data base.

Monkey ED50 data for SSVDT have been added to the primate data base.

Response strength and duration measurement systems have been ordered. First animals are being run on a prototype system.

In preparation for the brain graft work, we have developed a system by which we can produce selective radiogenic lesions of the hippocampus of newborn rats. We have a behavioral measure that correlates with this brain damage.

We have collected data that suggest that the peripheral nervous system may play a role in the production of opiate-induced and radiogenic behaviors.

We have shown a functional relationship between brain histamine systems and brain opiate systems. Both neurochemicals have relevance in the production of radiation-induced behaviors.

BEHAVIORAL SCIENCES DEPARTMENT

Group Title: Physiological Psychology Division

Objectives

To identify physiological mechanisms underlying performance degradation, ETI, conditioned taste aversions (CTA), and emesis.

To develop therapeutic approaches to prevent radiation-induced performance degradation, ETI, CTA, and emesis.

Current Status

We are determining the role of humoral factors in emetic response and conditioned taste aversions.

We are determining the role of neurotransmitters and neuromodulators (e.g., dopamine, endogenous opiates) in radiogenic performance decrements.

We are determining the role of stimulated Na^+ movement in neurons.

Future Goals and Directions

Assess the role of additional neurotransmitter systems (e.g., GABA, acetylcholine) underlying performance degradation and ETI.

Determine the role of ion movements in neurons.

Isolate and characterize blood-borne humoral factor(s) associated with radiation-induced emesis and conditioned taste aversions.

Determine how aging influences the response to radiation.

Accomplishments

We found that the movement of sodium ions across neuronal membranes is impaired after exposure to ionizing radiation, an effect that would disrupt normal excitatory processes.

We found that dopaminergic activity is reduced after exposure to ionizing radiation and correlates with the time course of ETI. This effect may reflect changes in motor activity.

We established that the area postrema, an important structure in the development of radiation-induced emesis and conditioned taste aversions, mediates toxic responses of emetic drugs and the radioprotectant WR-2721.

We found that the efficacy of gaseous anesthetics is not altered after exposure to gamma photons or neutrons. However, morphine-induced analgesia is enhanced after neutron irradiation under some conditions.

BIOCHEMISTRY DEPARTMENT

Group Title: Radiation-Induced Membrane Damage and Mechanisms of Altered Cell Function

Objectives

To describe the relationship between radiation-induced lysosomal hydrolase release and altered intracellular functions.

To assess the possibility that penetration of hydrolases into the nuclear region causes some of the acute and chronic postirradiation damage to the genetic machinery of the cell.

To describe mechanisms for the translocation of lysosomal hydrolases into the nucleus.

To attempt to block this process pharmacologically and thus prevent or minimize this aspect of radiation damage.

Current Status

We are studying a postirradiation rise in accessible, intracellular lysosomal hydrolase activity, which can be correlated with intracellular damage such as the rapid drop in the number of mitochondria.

We are finding that radiation also promotes permeability changes in several intracellular membranes, including the nuclear membrane.

Lysosomes are being shown by light microscopy to aggregate around the periphery of the nucleus following radiation.

Radiation-induced damage to the genetic machinery is being explained in terms of the hypothetical action of a variety of potent hydrolases.

Future Goals and Directions

Study the effect of radiation on lysosomal enzyme activity in cultured cells and cells derived from radiation-sensitive animal organs.

Identify the presence of lysosomally derived enzyms in highly purified preparations of nuclei extracted from irradiated cells.

Using light microscopy and specific staining techniques, confirm that these enzymes penetrate the nuclear envelope.

Correlate lysosomal enzyme activities in the nucleus with specific classes of damage to the genetic machinery.

Describe a mechanism for the radiation-induced release of hydrolases from lysosomes into the nucleus.

Accomplishments

Gamma irradiation (10 Gy) has been shown to promote an up to fivefold increase in the association of the lysosomally derived enzyme beta-glucuronidase, with highly purified nuclear fractions from cultured HeLa cells and rat spleen cells.

The degree of this association is dose-dependent from 2.5 to 10 Gy (gamma) for HeLa cells.

BIOCHEMISTRY DEPARTMENT

Group Title: Naturally Occurring Radioprotectors

Objectives

To study radioprotective factor(s) from a naturally radioresistant organism and to determine its efficacy as well as mechanism of action in preventing radiation damage.

To investigate the role of polyamines as exogenously administered in vivo radioprotectants, since evidence indicates that polyamines may protect cell-free DNA from X-ray denaturation.

To investigate the interrelationship of dietary elements, especially those with antioxidant properties (vitamins E, A, C, selenium, and other minerals) with respect to radioprotective properties.

To determine the relative importance of endogenous protective enzyme systems (superoxide dismutase, glutathione peroxidase, catalase) and endogenous nonprotein sulfhydryl compounds (reduced glutathione) in preventing radiation damage to cells and tissues.

To investigate the radioprotective properties of nontoxic monophosphate lipid A, the principal bioactive component of endotoxic lipopolysaccharide (LPS).

Current Status

Organisms known to be naturally radioresistant have poorly understood factors that provide this protection. We are determining whether there are potential medical applications for these substances.

The initial objective is to examine the effect of radiation on polyamines in rapidly dividing in vivo cell populations. Polyamines are important not only for normal growth of tissues but also appear to play a critical role in the recovery of tissues from injury.

The effects of radiation on nutritional status and, conversely, the effects of specific dietary elements on radiosensitivity are being studied.

The role of enzymes (superoxide dismutase, glutathione peroxidase, catalase) in radioprotection is being investigated.

Although LPS has been shown to be radioprotective, the toxicity of diphosphate lipid A (LAD) has prevented its use as a radioprotectant. However, one can now study the radioprotective properties of a recently prepared nontoxic monophosphate lipid A (LAM).

Future Goals and Directions

Evaluate the radioprotective properties of the two major components of the bacterial radioprotectant fraction in mice.

The interaction of a radioprotective drug, WR-2721, with endogenous polyamines will be studied. WR-2721 is structurally related to the polyamines and shows a striking resemblance to spermidine.

Develop a dietary regimen that will increase tissue levels of endogenous protective enzymes and glutathione, to optimize radioprotection.

Evaluate the effect of drugs on endogenous protective enzyme systems.

Investigate the hypothesis that lipid A may be acting on a target cell possibly located in the RES system, mediating the release of a radioprotective serum factor.

Accomplishments

An extract from D. radiodurans has been resolved into a 1000-dalton fraction that provides radioprotection when added to bacterial cells and mice, using reversed-phase high-performance liquid chromatography; this radioprotective fraction has been resolved into two major components.

Preliminary work has been centered around development of an HPLC method that can effectively separate and quantitate polyamines along with their acetylated derivatives.

Methods have been developed by gas chromatographic techniques for identifying the volatile products formed on the peroxidation of lipids in cell membranes. These will be applied to determine the role of endogenous protective systems in radioprotection.

Possible reasons for the radioprotection given by increased amounts of vitamins and minerals have been studied. Increased survival of irradiated mice fed a diet of high vitamin E and selenium may be due to increased levels of glutathione peroxidase in tissues.

Native endotoxin (LPS) as well as LAD and LAM have been shown to be radioprotective in mice. However, radioprotection does not correlate with the ability to stimulate hematopoiesis since LPS and LAD stimulated hematopoiesis but LAM did not.

BIOCHEMISTRY DEPARTMENT

Group Title: Chemical Radioprotection

Objectives

To obtain more effective radioprotective agents than are now available.

To determine the mechanisms of action of potential radioprotective agents in order to provide a rational basis for the design of new radioprotective drugs.

To determine the effectiveness of combinations of radioprotective agents and to develop new delivery systems for these drugs.

To determine effectiveness of combinations of radioprotectants and immune modulators.

To determine effects of general nutrition on radiation.

Current Status

WR-2721 is the most effective radioprotectant, but other factors being considered are its toxicity and side effects (nausea, hypotension). It does not protect the central nervous system.

A study is in progress on the subcellular distribution and mechanism of action of WR-2721. This study should prove useful in identifying the sites of action at the subcellular level.

Future Goals and Directions

Study the radioprotective activity in mice of various compounds in comparison and in combination with WR-2721 derivatives.

Investigate the possibility of using new drug delivery systems (e.g., micro-encapsulation).

Evaluate protection against GI, hematopoietic, and CNS damage, using various compounds.

Combine improvements in general nutrition, immunomodulators and radioprotectants to improve survival and increase measured enzyme activities after radiation.

Accomplishments

We have studied the effects of potential radioprotectants and/or immunomodulators on cell-mediated immunity in irradiated mice; this was determined by measuring changes in delayed-type hypersensitivity.

We have attempted to correlate the potency of various radioprotective drugs with their ability to alter intracellular glutathione peroxidase activity after radiation.

Improvements in general nutrition have increased survival and glutathione peroxidase activity after radiation.

WR-2721 has been shown to be detrimental in terms of behavior both before and after exposure to ionizing radiation.

BIOCHEMISTRY DEPARTMENT

Group Title: Mast Cell in Radiation and Combined Injury

Objectives

To examine several classes of drugs that could serve as potential inhibitors of mast cell degranulation (antianaphylactics) for their effect on both intracellular free-calcium levels and histamine release.

To detemine the effect of these antianaphylactic compounds on radiation injury/survival and on wound repair mechanisms.

To elucidate the role of the high-affinity Ca^{+2}, Mg^{+2}-ATPase (calcium pump) in the secretion process and to study the effects of calcium antagonists on enzyme activity and the secretion process.

To evaluate immunoregulatory compounds that increase radiation survival for their effect on mast cell degranulation.

Current Status

Investigations of calcium metabolism in mast cells using ^{45}Ca as a tracer have been completed.

The toxic components of a calcium fluorescent probe (quin-2) have been identified, and fluorescent detection of intracellular calcium changes occurring during secretion has been measured. Fluorescent detection will be the method of choice for future studies.

Inhibition of the calcium pump by calmidazolium is being found to result in histamine release in isolated mast cells.

Future Goals and Directions

Initiate studies with Fura-2 as a less toxic and more sensitive intracellular calcium fluorescent indicator.

Study the mechanisms of mast cell activation by glucan in vivo in complement-deficient and complement-depleted animals.

Evaluate several antianaphylactic drugs and mediator-blocking drugs for their effectiveness in ameliorating symptoms and survival patterns following radiation and/or combined injury.

Accomplishments

Glucan, an immunomodulating compound that is effective in increasing radiation survival, causes an immediate and significant mast cell degranulation in vivo. Kinetic studies of the release pattern revealed a prolonged stimulatory effect on the mast cell, which is maintained up to 30 days following a single injection.

Techniques have been perfected for monitoring alterations in intracellular free-calcium concentrations in stimulated mast cells using the recently synthesized intracellular fluorescent probe quin-2. Optimal doses with minimal mast cell toxicity have been determined.

Perigranular and plasma membranes have been isolated from mast cells, and the calcium pump has been characterized and localized exclusively to the plasma membrane. The apparent molecular weight of the calcium pump in situ has been determined using radiation inactivation and target size analysis.

BIOCHEMISTRY DEPARTMENT

Group Title: Radiation and Combined Injury: Circulating Mediators

Objectives

To identify circulatory factors and hormonal mediators that may have causal roles in the sequelae of radiation and/or combined-injury performance decrement and incapacitation.

To examine trauma-induced alterations in mediator content. Correlate the kinetics of tissue, plasma, and urinary levels of mediators in trauma with the cellular disturbances produced in the hematopoietic system. Determine if pharmacologic manipulation of endogenous mediator synthesis provides protection or enhances recovery from injury. Correlate the kinetics of pharmacologic intervention with the course of myeloid and lymphoid recovery.

Current Status

The biological test systems include invasive (blood, CSF, biopsies) and noninvasive (urine) sampling. Mediators with synergistic and/or modulatory relationships, prostaglandins, thromboxane, leukotrienes, cyclic nucleotides, complement, ACTH, and cortisol/corticosterone are being examined as potential indicators of radiation/combined injury.

We are establishing the role of these vasoactive substances in radiation-induced damage and seeking information on how radioprotectors modulate chemical mediators and messengers. We are evaluating the possibility of interventions by various types of drugs to alleviate radiation-induced injury.

Mediators will be studied in relation to dose of gamma or enhanced neutron radiation in models of different species (monkey, dog, mouse, guinea pig). Interspecies differences will be evaluated in terms of potential extrapolation to man.

Future Goals and Directions

The mechanisms of radiation-induced alterations in arachidonate metabolism will be examined by pharmacologic blockade of key enzymatic processes. Steroid (dexamethasone) will be used to study the mechanism of phospholipase A_2 activity on the release of fatty acid substrates from phospholipids. The nonsteroidal anti-inflammatory drugs (NSAID) indomethacin and aspirin (which directly inhibit cyclooxygenase enzyme activity) will be studied to examine the contributions of the products of this enzyme to the inflammatory response. The calcium channel blockers verapamil and nifedipine will be used to inhibit acyl hydrolase activation and prevent arachidonate release.

Prostaglandin and leukotriene release in a radiation scenario will be studied in isolated peritoneal and bronchial lavage macrophages from mice. This cell type has been selected because the role of prostaglandins in macrophage cell function is important, the macrophage takes precedence as a major prostaglandin-producing entity, and the macrophage is actively involved in radiation-induced inflammation.

Accomplishments

Increased levels of prostaglandins and glucocorticosteroids have been implicated in the modulation of the lymphomyelopoietic system after trauma. We have demonstrated that these substances are released in mice after sublethal single or combined traumas.

In the mouse model, the reliability of laboratory measurement of mediators as indicators of radiation insult suggests that the concentration of urinary PGE_2 may be useful in the estimation of whole-body radiation exposure levels, over a wide range of radiation doses. Kinetics of circulating PGE_2 levels in radiation, trauma, or combined injury also implicate this mediator as a potential candidate as modulator of the immunological responses.

We have examined the role of the classic pathway of complement activation using C4-deficient guinea pigs. Our results do not support a fundamental role for the functional activity of the classical pathway in survival from acute radiation exposure; however, it may participate in the immunoregulation of arachidonic acid metabolism/excretion in the radiation-damaged kidney.

Ongoing studies with the rhesus monkey suggest a role for the prostaglandins in the regulation of gastric secretion and emptying and also the mechanism of the early radiation-induced suppression of acid output. Results suggest PGE_2 and PGI_2 may be responsible for the immediate suppression of acid output, while $PGF_{2\alpha}$ may be involved in the delayed radiation sickness.

Group Title: Hematopoietic Stem Cell Isolation

Objective

To isolate a pure population of functional hematopoietic stem cells that can be used (a) to study graft versus host free bone-marrow transplants in irradiated individuals and (b) to study directly, using recombinant DNA techniques, the effects of ionizing radiation on the hematopoietic stem cell.

Current Status

AFRRI has available to its investigators a state-of-the-art fluorescence activated cell sorter for isolating hematopoietic immune cell populations. Using this technique, we have developed a method for purifying hematopoietic stem cells.

Future Goals and Directions

Now that we can generate nearly pure populations of hematopoietic stem cells, the following avenues of research will be attempted in the coming fiscal year:

1. Extrapolation of this stem cell isolation technique for the purpose of studying marrow transplantation free of graft versus host disease in a large-animal model.

2. Assess and modify this technique to the point where it can be used as a biological dosimeter; that is, can we measure the number of surviving hematopoietic stem cells in the postirradiated individual using flow cytometry?

3. Using the technique of recombinant DNA, study directly (at the molecular level) the effects of radiation on isolated hematopoietic stem cells and investigate the nature of those molecules regulating hematopoietic self-renewal and differentiation in the postirradiation animal.

Accomplishments

Substantial progress was made toward purifying (100 X) the hematopoietic stem cell. This was accomplished by using the new fluorescent protein (Phycoerythrin) and the development (in BICI) of a new technique to conjugate this protein to Fab fragment of monoclonal antibodies. This allowed us to perform two-parameter immunofluorescent sorting using a single laser at sort rates of 7000 cells per second. Using this technique, nearly pure rat hematopoietic stem cell populations of 100,000 cells can be generated within 8 hours.

BIOCHEMISTRY DEPARTMENT

Group Title: Mucosal Immune System and Radiation/Combined Injury

Objectives

To investigate the effect of radiation on the IgA immune system (Peyer's patches, mucosal immune system).

To investigate methods by which the IgA arm of the immune system may be manipulated and/or modulated in order to reestablish immune function in irradiated individuals with other severe injuries.

Current Status

The effects of radiation on the IgG arm of the immune system are well known.

There is little knowledge about the effects of radiation on the IgA arm of the immune system.

In order to investigate radiation damage to the mucosal immune system and mucosal microenvironment, a rat chimeric animal model (using rat strains Lewis, NBr, M520, and BUF) is being set up. This animal model will allow us to trace the regeneration of specific lymphocytes from bone marrow to the mucosal immune system, and eventually to determine what effects radiation has on the idiotypic determinants of the immune system.

Future Goals and Directions

Radiation effects on the mucosal immune system will be evaluated.

Specific subpopulations of mucosal lymphocytes will be isolated from irradiated animals to determine which cells are functional after radiation exposure.

Procedures will be determined by which the immune competency of the mucosal immune systems may be restored in combined-injury victims.

An assay system will be developed that is capable of analyzing viable lymphocyte subpopulations in lymphoid tissue from irradiated animals. The effect of radiation on the gut-associated and mucosal-associated lymphoid tissue (GALT and MALT) will be investigated, starting with Peyer's patches, intraepithelial lymphocytes, mesenteric lymph nodes, and lamina propria lymphocytes.

Accomplishments

It has been concluded that after 150 roentgens of ionizing radiation, Peyer's patch lymphocytes from rats are damaged, and regeneration of these lymphocytes is extremely slow, compared to the IgG arm of the immune system.

It has been found that the spleen is not required for a functioning IgA system.

Assays designed to test the function capability of lymphocytes isolated from the mucosal immune system of irradiated rats suggest that the regenerating lymphocytes have normal function capacity, at least on a short-term basis. At the present time, alteration in idiotypic determinants of lymphocytes has not been studied.

BIOCHEMISTRY DEPARTMENT

Group Title: Radiation Effects on Bone and Bone-Marrow Development

Objective

To study the effects of ionizing radiation on the function and development of bone and bone marrow.

Current Status

We are developing a method of inducing bone and bone-marrow formation in young rats.

We are studying the effects of radiation on cellular targets in bone and the underlying mechanisms.

We are studying radiation effects on the interrelationship of bone and bone-marrow developments.

Future Goals and Directions

Determine the RBE of the hematopoietic microenvironment, using the model for endochondral bone and bone-marrow development. Develop methods by which radiation damage may be prevented or repaired.

Isolate and characterize growth factors that may be responsible for bone and bone-marrow self-renewal. To evaluate these factors in reversing the effects of radiation and combined injury on bone development, remodeling, and fracture healing.

Accomplishments

Effects of radiation on the critical stage of radiation-induced bone formation and mineralization were studied.

We found that sublethal irradiation delayed the differentiation of cartilage and bone by impairing progenitor cell proliferation.

We determined that the cells that comprise the microenvironment of the hematopoietic system are sensitive to ionizing radiation.

When mouse bone marrow was incubated in medium supplemented with a 4M Gnd-HCl extract of rat demineralized bone powder, the survival of hematopoietic stem cells as determined by CFU-S content was 25%-36% greater than in marrow cultured in medium not supplemented with the extract. In addition, marrow cultured in media supplemented with various collagen fractions did not enhance survival of CFU-S in vitro. Partial purification by gel filtration of the 4M Gnd-HCl extract showed that the active factor had a molecular weight of less than 50,000 daltons. The manner in which this factor interacted with hematopoietic stem cells is not known. Apparently the developmental cascade initiated by these factors is radiosensitive. The role of these factors in the recovery of bone and bone marrow following irradiation will continue to be investigated.

EXPERIMENTAL HEMATOLOGY DEPARTMENT

Group Title: Stem Cell Physiology Program

Objective

To delineate the mechanisms involved in the regulation of the processes of proliferation and differentiation of the hemopoietic stem cell and its committed progeny.

Current Status

Reliable in vitro assays are being established for multipotent hemopoietic cells from murine, canine, and monkey tissue.

Separation techniques, based on physical and immunologic properties of hemopoietic cells, are being developed singularly and in combination. Techniques being used are rosetting with SRBC, and soybean agglutination in combination with density separation and counterflow elutriation. This effects the depletion of unwanted T-lymphocytes while concentrating the stem cells.

Future Goals and Directions

Establish reliable assays for hemopoietic stem cells in the canine and primate.

Use monoclonal antibodies and specific growth factors to delineate cellular relationships in murine and primate hemopoiesis in normal and irradiated animals.

Establish a long-term culture system for primate bone marrow.

Initiate use of monoclonal antibodies against cells of the murine, canine, and primate hemopoietic systems to remove specific undesirable cell types from the total population as well as positively select for stem cell population.

Use autologous bone-marrow transplant in the canine and primate to identify a functional pluripotent stem cell capable of long-term reconstitution.

Accomplishments

An in vitro assay for a multipotent stem cell has been established in the primate system.

Using monkey bone marrow, the presence of this cell type in various cell fractions following counterflow centrifugation-elutriation has correlated with those fractions that promote long-term survival.

Immunologic separation of primate bone-marrow cells using sheep-red-blood-cell and soybean-lectin receptors on T-lymphocyte populations has concentrated the populations of hemopoietic progenitor cells twofold to fivefold.

A model of autologous bone-marrow transplantation has been established in the primate. Whole bone marrow and separated bone marrow have been transplanted, with good results in terms of survival, engraftment, and reconstitution of peripheral elements.

EXPERIMENTAL HEMATOLOGY DEPARTMENT

Group Title: Neutron-Gamma RBE

Objective

To determine the relative biological effectiveness (RBE) of mixed fission neutron:gamma radiation for hemopoietic and gastrointestinal effects in a large-animal (canine) model.

Current Status

We are establishing the LD50/30 for the canine after exposure to AFRRI TRIGA (0.8 MeV avg.) neutrons (4:1 neutron:gamma) at 1.48 Gy.

We are determining that the neutron effectiveness for hemopoietic death in the canine for 0.8 MeV neutrons relative to cobalt-60 gamma irradiation is approximately 1.63.

This neutron effectiveness is also being reflected in the sensitivity of specific cell types in the target tissue, the bone marrow.

We are finding that the canine GI system is also very sensitive to neutrons. The neutron effectiveness for the GI system may be as high as 5.0, when using LD50/7 as an index.

Future Goals and Directions

Determine depth-dose relationships and target organ dose in a canine mixed neutron:gamma radiobiology model.

Determine the LD50/30 and LD50/7 in the canine for unilateral and partial-body neutron irradiation. Extend the exposure mode to pulse irradiation.

Correlate the above with assays for functional hemopoietic cells and epithelial cells in target organs of bone marrow and gastrointestinal tissue.

Accomplishments

Canines have been unilaterally exposed to a mixed neutron:gamma dose range of 1.50 to 2.50 Gy. LD50/30 values have been placed at 2.25 Gy, relative to approximately 1.48 Gy for bilateral whole-body exposure.

It has been found that early death of these animals indicates greater gastro-intestinal involvement, in addition to marrow depletion.

We have determined that a bilateral mixed neutron:gamma dose of 2.00 Gy is over 90% lethal. Autologous bone marrow transplant ensures only 50% survival, again indicating greater involvement of damage to the GI system with neutron irradiation.

EXPERIMENTAL HEMATOLOGY DEPARTMENT

Group Title: Post Radiation Immunoenhancement

Objective

To enhance postirradiation recovery in hemopoietic stem and progenitor cell populations, and to protect from opportunistic infection.

Current Status

New hemopoietic stem cell assays are being tested for reliablity. D_o and recovery parameters are being determined.

Various immunomodulators are being tested for their ability to enhance the recovery of hemopoiesis following irradiation. These include glucan, thymosin, and IL-1, and selected agents from the Biological Response Modifiers Program (NCI, Frederick, MD).

Glucan is proving to be very effective in stimulating hemopoiesis when given before and immediately after irradiation.

Glucan is being tested in combination with agents such as WR-2721, antibiotic regimens, and other immunomodulator therapy.

Future Goals and Directions

Determine the ability of the immunomodulator glucan to enhance hemopoiesis in the canine and primate.

Study the events associated with thymosin protection from infection and immune cell recovery in an attempt to define the common mechanism.

Determine the efficacy of combining agents such as glucan or thymosin, antibiotics, and immunoglobulins in the large-animal radiobiology and sepsis models.

Determine effectiveness of IL-1 on postirradiation resistance to infection.

Study the mechanism of IL-1-induced radioprotective events.

Determine what other lymphokines are important in radioprotection.

Accomplishments

We found that both particulate and soluble glucan enhance hemopoietic repopulation in sublethally irradiated mice and significantly enhance survival in otherwise lethally irradiated mice.

Both the glucan particulate (P) and soluble (F) forms were shown to enhance the survival of mice to radiation doses of 9.0 to 11.0 Gy prior to exposure. In this mode, the glucan P form was shown to be more effective than the glucan F. However, when the glucan was administered after the radiation exposure, glucan P was not shown to enhance survival, whereas glucan F enhanced survival. The survival was not as great as when the glucan was administered prior to irradiation.

We found that thymic hormones administered after irradiation protect radiation-immunocompromised mice from opportunistic infections with P. aeruginosa.

We determined that thymic hormones accelerate the rate of recovery of immunocompetent cells.

EXPERIMENTAL HEMATOLOGY DEPARTMENT

Group Title: Control of Sepsis in Combined-Injury Models

Objective

To prevent and treat radiation effects, including radiation-induced hemopoietic dysfunction, and the medical and surgical therapy of combined effects.

Current Status

Small-animal and large-animal (canine) models for sepsis are being established for investigating the mechanisms of cellular dysfunction following irradiation.

The irradiated canine model for peritoneal, gram-negative sepsis is being characterized relative to lethality, hemopoietic and immune responses, and hemodynamic status.

Small-animal models are being used in combination with trauma models such as wounds and thermal injury.

Monoclonal antibodies to lymphocyte subpopulations are being used to decipher changes in specific subpopulations following irradiation and sepsis.

Specific mediators such as interferon, interleukin, plasminogen activator, and acute-phase reactants are being investigated relative to the cellular inflammatory response following infection in normal and irradiated animals.

Future Goals and Directions

Establish immunologic parameters to be measured in the various models of radiation, trauma, and combined injury. These include lymphocyte populations, macrophages, and mediators of inflammation involved in both systemic and mucosal immune systems.

Define the dynamics of microbial changes after radiation, trauma, and combined-injury regimens.

Explore the timing variable in the interval between exposure to radiation and trauma.

Evaluate "biological response modifiers" modulation of immune suppression following radiation and trauma.

Utilize the canine model of peritoneal sepsis for assessing avenues of therapy.

Accomplishments

The large-animal model of canine peritoneal sepsis has been established as a valid model of human gram-negative hyperdynamic sepsis.

We have determined that Escherichia coli bacteria (dose-dependent) can be administered within 4 hours or 6 days after a sublethal dose of radiation (1.50 Gy cobalt-60) without inducing lethality. Fluid therapy is essential. Radiation of 1.5-2.0 Gy enhances susceptibility to E. coli infection.

We determined that the dose of radiation (1.50 Gy cobalt-60) does not impair the ability of granulocytes to chemotax, phagocytose, or kill bacteria.

A militarily relevant mouse model of combined injury was developed for identifying (a) circulating mediator and immune responses after injury, (b) survival responses after small-dose bacterial challenge, and (c) agents that show therapeutic promise in combating infectious complications.

We found that the levels of a presumptive circulating mediator associated with sepsis, PGE_2, were increased during the time frame (5-14 days) when animals died with infections.

Humoral immunity (opsonic proteins), particularly IgG, was found to be severely depressed (20% of controls) during the time period when death with infections occurred (5-14 days after combined injury).

EXPERIMENTAL HEMATOLOGY DEPARTMENT

Group Title: Cellular Radiobiology Division

Objectives

To investigate the molecular basis for cellular radiosensitivity and its modification.

To examine cell cycle influences on radiosensitivity using mammalian cells in synchronous growth in vitro.

To study three major research themes:

1. DNA lesions induced by ionizing radiation.
2. Cellular expression and modification of lesions in DNA.
3. Response at the tissue level to radiation injury.

Current Status

1. Characterization of Lesions in DNA

Hydroxyl Radical Model. A model for the study of indirect radiation effects in cellular studies, using hydrogen peroxide (H_2O_2) in a Fenton-type reaction, is being extended to included cell-free DNA model systems.

DNA Base Damage. DNA base damage characterized by gas chromatograph-selective ion mass spectroscopy is under way for DNA repair enzymology studies.

Effects of DNA Damage on Semiconservative DNA Synthesis. Methods are being developed to produce controlled DNA lesions upstream to the replication fork (i.e., prereplicative lesions) in synchronized cells to determine their incluence on semiconservative DNA synthesis.

2. Cellular Expression and Modification of Lesions

Modification of Sulfhydryl Concentration. The link between the level of sulfhydryl compounds within mammalian cells and their inherent radiosensivity is under investigation. The glutathione content of mitotic cells is being correlated with cellular radiosensitivity parameters. Modification of sulfhydryl levels with drugs that either bind/chelate sulfhydryls or block glutathione synthesis is being used to investigate the importance of thiols in radiosensitivity.

Enzymology of Base Damage Repair. Microassays are being developed for the study of DNA base damage repair and its regulation in cells. Radiolytic products of DNA that are suitable for removal by cellular enzymes are being identified.

3. Response of Radiation Injury at the Tissue Level

Control of Cell Proliferation in Hematopoietic Tissue. Study the control of cell proliferation in hematopoietic tissues, with emphasis on verifying the Fe ion data and extending to higher and lower LETs.

Radiobiology of Hexose Transport Regulation. Study radiation effects on gene expression based on the hypothesis that inhibited repair of DNA damage should exacerbate radiation effects, measured as the induction of hexose transport, if genetic expression is involved.

Accomplishments

1. Characterization of Lesions in DNA

Hydroxyl Radical Model. The production of 10 Gy equivalent single strand break yields by H_2O_2 at 0°C were found not to be lethal suggesting an aggressive DNA repair system within the cell. Cell cycle variations in H_2O_2-induced cell killing at 0°C with marked S-phase sensitivity was observed. In a cell-free model system, hydroxyl radicals were monitored by the production of ethylene gas during the oxidation of methional.

DNA Base Damage. DNA base damage studies have resulted in recognizing and quantifying thymine glycol in DNA suitable for enzymology experiments.

Effects of DNA Damage on Semiconservative DNA Synthesis. The effect of lesions in DNA downstream to the replication fork were studied. Strand break type lesions were introduced in 3%-5% of the genome through pulse labelling with a thymidine analog (BrdUrd), followed by exposure to 313 nm light. Chain breaks produced within 90 minutes of the growing fork, which is the interval required for complete maturation of replicons, halted both initiation and elongation components of DNA synthesis.

2. Cellular Expression and Modification of Lesions

Modification of Sulfhydryl Concentration. An HPLC laboratory was established to quantify various nonproteir sulfhydryl compounds at the nanomolar level. Preliminary studies indicate that mitosis is associated with a large increase in the concentration of glutathione, the primary nonprotein sulfhydryl.

Enzymology of Base Damage Repair. Modified DNA bases other than thymine glycol, produced by ionizing radiation, were identified following osmium tetroxide treatment of DNA.

Repair Patches Formed During Repair of DNA Damage. L5178Y-S mouse cells, which are sensitive to X rays but possess normal sensitivity to UV light, have been shown to effect repair at a rate of 1-2 lesions per 1.0×10^8 daltons per day. After 24 hours, L5178Y-R cells, which are resistant to X rays but extremely sensitive to UV light, effect no repair and, in fact, no DNA synthesis.

Repair Patches Formed During Repair of DNA Damage. Improvements in a technique to measure the removal of lesions in parental DNA (i.e., BrdUrd substitution/313 nm photolysis) now permit sensitive measurements of cell-mediated "cut and patch" repair. The variation in radiation sensitivity of two related mammalian cell lines is being correlated with their ability to remove DNA damage by patch repair.

3. Response of Radiation Injury at the Tissue Level

Control of Cell Proliferation in Hematopoietic Tissue. The effect of radiation quality (i.e., HZE particles of various energies) on the injury of dormant marrow stem cells, measured by their ability to be induced to proliferate, is under way.

Radiobiology of Hexose Transport Regulation. The feasibility of studying the effects of radiation on gene expression in an in vitro model system is being addressed. The induction of glucose transport in plateau-phase cultures of porcine kidney cells is being developed as a potential genetically controlled expression event.

Future Goals and Directions

1. Characterization of Lesions in DNA

Hydroxyl Radical Model. Develop a hydroxyl radical model (e.g., using a Fenton-like reaction) for studying indirect radiation effects, measuring hydroxyl radical yields, identification of types of DNA base damage, and correlation of lesion repair kinetics with cell recovery.

DNA Base Damage. Continue the development of a reliable and sensitive assay for DNA base damage.

Effects of DNA Damage on Semiconservative DNA Synthesis. Investigate the effects of DNA damage upstream to the replication fork on semiconservative DNA synthesis.

2. Cellular Expression and Modification of Lesions

Modification of Sulfhydryl Concentration. Study the importance of sulfhydryl compounds within mammalian cells and radiation sensitivity with particular attention to the influence of the cell cycle and hormonal effects (i.e., PGE_2).

Enzymology of Base Damage Repair. Continue development of microasssays for DNA base damage enzymology. Determine the regulatory mechanism for enzyme levels and define cell cycle influences.

Repair Patches Formed During Repair of DNA Damage. Correlate the influence of repair patches formed during repair of DNA damage with the recovery of DNA synthesis.

3. Response of Radiation Injury at the Tissue Level

Control of Cell Proliferation in Hematopoietic Tissue. A threshold dose is necessary to induce proliferation of bone-marrow cells by sparsely ionizing radiation. Preliminary studies have shown that the cycling of dormant (i.e., noncycling) stem cells occurs in some animals following irradiation with iron particles at 600 MeV/amu.

Radiobiology of Hexose Transport Regulation. Exposure to ionizing radiation or cyclohexamide blocks induction of glucose transport for several days. The induction block was shown to be dose-dependent (D_0 of ~ 25 Gy) and could be clearly differentiated from radiation-induced cell-killing effects.

PHYSIOLOGY DEPARTMENT

Group Title: Cellular Physiology Division

Objectives

To determine if exposure of cultured endothelial cells to ionizing radiation results in increased vascular permeability or altered cellular synthetic mechanisms.

To evaluate the mechanisms by which radiation damage affects the cell function of a number of cell types, by means of alteration of the membrane transport mechanism.

To determine the effects of radiation on an animal's host-defense mechanisms, by evaluating the role of the macrophage in combating infections and how macrophage function is affected by radiation.

To develop methods to image living cells and make quantitative measurements of fluorescence in single living cells. The goal is to be able to quantitate the responses of cell volume and intracellular ions (H, Ca, Na, Cl, K) to physiological stimuli such as effects of radiation.

Current Status

Endothelial cells from bovine aortas and cerebral tissue are being grown in tissue culture.

Sodium-dependent glucose transport and the Na-H exchange in kidney epithelial cells are being evaluated.

Cellular properties of the human peripheral blood-derived macrophage, mouse macrophages, and macrophage-like tissue culture cell lines are being evaluated, using biochemical and electrophysiological techniques.

The imaging system is in the process of being developed.

Future Goals and Directions

Endothelial cells grown from a number of sources will be evaluated as to their structure, function, and radiation sensitivity.

The effect of radiation on the Na-H exchange system, an integral step in cell proliferation, will be examined in epithelial cells.

Macrophages from a number of sources will be evaluated for their secretory, phagocytic, and chemotactic abilities following radiation. The biophysical properties of these cells will be investigated along with the effects of radiation on these properties.

Endothelial and epithelial cells will be examined to determine the effects of physiological stimulation and radiation on permeability, levels of intracellular messengers, and cell volume.

Accomplishments

It had been determined that radiation alters the regulation of sodium-mediated glucose transport in kidney epithelial cells.

Using patch clamp methodology, single ion channels in the membrane of macrophages have been characterized. In addition, changes in ionic conductances that occur during the maturation sequence of the human macrophage have been documented.

Functional studies have indicated that macrophage-like cells exposed to radiation increase their phagocytosis and superoxide release, and develop a more activated state.

The radiation sensitivity of bovine endothelium in terms of thymidine incorporation and cellular protein content has been determined.

PHYSIOLOGY DEPARTMENT

Group Title: Neurophysiology Division

Objectives

To study the cellular changes that occur with radiation injury and how radiation affects excitable membrane properties.

To study the actions of compounds that might mediate radiation-induced changes in neuronal excitability.

To study the mechanism of radiation-induced fatigue.

To investigate how radioprotectants directly affect neuronal properties and modify susceptibility to radiation.

Current Status

Several radiation-released compounds and the radioprotectant DTT are being studied, and their effects on hippocampal neuronal integration are being determined using neuronal brain slices.

A computer-assisted system is currently in operation assessing the synaptic quantal content of neurotransmitter release in irradiated and matched nonirradiated synaptic junctions.

Effects of radiation on electrophysiological properties of the hippocampus are being evaluated in the brain slice preparation.

Future Goals and Directions

Individual ionic mechanisms by which synaptic control is affected by radiation-released compounds and by ionizing radiation will be found.

The use of the neuromuscular junction of the mouse and rat will be pursued to evaluate the effects of radiation on prolonged fatigue and performance decrement.

Interactions of the various radiation-released compounds with each other and with radioprotectants will be examined.

Accomplishments

Radiation has been shown to alter characteristics of neuronal sodium channels.

One radiation-released compound changes neuronal excitability by modulating a potassium current. Other possible mediators of radiation damage were found to decrease neuronal excitability by depressing synaptic mechanisms and by impairing action potential generation.

The radioprotectant dithiothreitol has been found to transiently increase neuronal excitability through synaptic mechanisms.

PHYSIOLOGY DEPARTMENT

Group Title: Gastrointestinal Physiology (Subgroup of General Physiology Division and Cellular Physiology Division)

Objectives

Sublethal doses of radiation produce nausea, vomiting, and diarrhea, while supralethal doses result in loss of fluid, electrolyte loss, and death.

To study the effects of ionizing radiation on intestinal electrolyte transport at the cellular level in an effort to elucidate the mechanism of radiation-induced diarrhea.

To determine if part of the gastrointestinal syndrome is due to complications and alterations in gastrointestinal blood flow following radiation.

Current Status

Programs are being established to assess sodium, chloride, and potassium transport and their regulation across isolated gastrointestinal mucosa from both irradiated and nonirradiated animals in vitro.

Measurements of intestinal blood flow are being made in the ileum of dogs from both irradiated and sham-irradiated animals.

Future Goals and Directions

Isotope fluxes, short-circuit currents, and standard electrophysiological intracellular recording techniques will be used to evaluate alterations in cell membrane permeabilities and intracellular ionic activities.

The role of Ca and pH as intracellular triggers that initiate the loss of fluid and electrolytes associated with diarrhea as a result of radiation will be evaluated.

Accomplishments

It has been determined that cobalt-60 causes significant alterations in cellular intestinal electrolyte transport 24 to 96 hours postradiation, characterized by a stimulation of chloride secretion and a decrease in responsiveness to both secretory and absorptive stimuli.

Preliminary studies indicate a role of calcium in regulating the various membrane conductances that may be related to fluid and electrolyte losses.

Changes in blood flow in the gastrointestinal system have been evaluated in the presence of pharmacological agents, including antihistamines.

We have determined that histamine blockers alter the initial postradiation increase in intestinal blood flow and the postradiation change in hematocrit measured in the gastrointestinal vascular system. The initial rise in blood pressure, probably due to histamine-mediated release of catecholamines from the adrenal gland, was abolished with antihistamines. The hypotensive phase was also altered with antihistamines.

PHYSIOLOGY DEPARTMENT

Group Title: Cardiovascular Physiology (Subgroup of General Physiology Division)

Objectives

Acute ionizing radiation produces a transient hypotension followed by a complete cardiovascular failure similar to that seen in circulatory shock.

To evaluate the effect of radiation-induced vascular hypotension of visceral organs, using intestinal vasculature as a model system.

To evaluate the production of histamine and other toxic by-products released by radiation to determine if they induce a circulatory shock taste.

To study the loss of cardiovascular reserve following radiation, which may contribute to fatality.

Current Status

Model systems are being used to evaluate cardiovascular dysfunction in various visceral organ systems. These include isolated gastrointestinal systems in the rat and the dog.

Cerebral vascular studies are under way to evaluate the changes in blood flow of individual areas of the central nervous system following whole-body and localized radiation.

Circulating radiation-released toxic elements are being evaluated for their mechanisms of action.

Various blockers are being evaluated to determine their radioprotecting potential.

Future Goals and Directions

Continue to evaluate the effects of antihistamines on intestinal blood flow in an attempt to evaluate the effect of histamine released as an indirect radiation insult.

Correlate radiation-induced cerebral ischemia with *in vivo* extracellular electrophysiological recording (EEG).

Accomplishments

Findings indicate that radiation-induced hypotension in the beagle was accompanied by increased intestinal blood flow.

H_1 and H_2 histamine blockers temporarily prevented the postirradiation increased blood flow and hemacrit.

A decrease in plasma glucose following the first hour postirradiation was demonstrated in beagles.

Postradiation regional cerebral blood flow has been measured in six areas of the primate brain and associated with early transient incapacitation. Radiation-induced cerebral ischemia has been prevented by infusion of selected chemical agents such as allopurinol and disodium cromoglycate.

Group Title: Molecular Radiobiology Division

Objectives

To study metabolic changes in single cells that occur with radiation injury.

To develop a fundamental understanding of the role of superoxide and hydroxyl radicals in mediating DNA damage and elucidate the importance of metal ions in catalyzing the reactions.

To develop a sensitive and quantitative fluorescence assay for hydroxyl radicals in cellular systems.

To quantitate the role of single strand breaks in the production of potentially lethal double strand breaks for irradiated aqueous suspensions of DNA both in the presence and absence of drugs and proteins.

To quantitatively assess the damage by ionizing radiation on solid films of oriented DNA.

To study the interaction of radioprotectants with DNA.

Current Status

Initial measurement using high-resolution phosphorous NMR on single cell suspension of yeast is indicating that ionizing radiation alternates the intracellular polyphosphates.

The EPR spectrum of neutron irradiation of oriented films of calf thymus DNA at 77 K is being found to be different, depending on the direction of the incident neutrons with respect to the DNA fiber orientation.

Double strand circular 0X 174 DNA covalently binding with benzo(a) pyrene diol epoxide has been shown to produce single strand breaks. Preliminary evidence is indicating that free radicals produced by ionizing radiation interact primarily at the same loci as the diol epoxide.

Flow cytometric studies of polymorphonuclear leukocytes is indicating a sensitivity to hydrogen peroxide at concentration of less than 1 μmolar.

The electron transfer reaction sequence in gamma-irradiated nuclei acids to intercalating antitumor agents and the subsequent production of superoxide is being measured.

Future Goals and Directions

Assess the role of high-energy phosphates in DNA damage following ionizing radiation.

Quantitate the types and amounts of free radicals produced in neutron-irradiated DNA films.

Sequence studies of DNA to elucidate differences and similarities between drug and ionizing radiation interaction with supercoiled DNA.

Quantitate the production of hydroxyl radicals in irradiated cells using fluorescence methods.

Investigate the role of conformation on radioprotectant interactions with DNA.

Investigate the role of superoxide and hydroxyl radicals in subcellular synaptosomal fragments on lipid peroxidation using spin trapping methods.

Accomplishments

Phosphate NMR baseline studies of single cell suspension of yeast have been completed.

The in vivo metabolic rate of dephosphorylation of the radioprotectant WR-2721 in mice has been measured by the noninvasive technique of nuclear magnetic resonance.

The electron transfer from the DNA intercalating agent adriamycin to dissolved oxygen has been measured by EPR spin trapping of the superoxide formed.

The survival response and potential lethal recovery following gamma irradiation of three rad mutants of yeast, each of which exhibits a different DNA repair mechanism, have been characterized.

Group Title: Physical Dosimetry

Objectives

To apply existing state-of-the-art physical dosimetry methodology to radiobiology research at AFRRI.

To develop new radiation dosimetry approaches,such as nanodosimetry and microdosimetry, which are fundamentally related to biological response. Apply these approaches to define radiation quality and RBE of laboratory radiation fields as well as those to which humans may be exposed, i.e., nuclear battlefield.

To study the radiation environments that may be produced by nuclear detonations. Determine the relevance of AFRRI radiation fields for radiobiology research to the case of human radiation exposure on a nuclear battlefield. Develop a rationale for dosimetric extrapolation from monkey to man.

To determine the accuracy and reliability of RADIAC devices (dosimeters) planned for use by the Military Services for nuclear detonations and fallout. Define the role those devices can play in medical management of mass radiation casualties, given the various physical limitations such as energy response, directionality, and orientation, which affect their accuracy.

Current Status

A major upgrade of the AFRRI radiation dosimetry program has been 90% completed.

Calculational approaches to microdosimetry are proceeding on schedule (50% completed), while experimental measurements of linear energy spectra have been delayed (25% completed) due to staffing shortages.

Pilot experiments on U.S. Army nuclear radiation dosimeters have been completed in AFRRI radiation fields, and further evaluations at ORNL are being planned.

Future Goals and Directions

Continue studies of physical dosimetry in large-animal models used for radiobiology research, especially canine and primate. Place emphasis on dosimetry and microdosimetry of bone marrow in support of research on hematopoietic damage studies.

Obtain further information on radiation fields to which humans may be exposed in future military operations, and on the local dose deposition patterns produced by those radiations. Apply this information to the design and analysis of radiation fields used for radiobiology research.

Apply microdosimetry and track structure analysis to the prediction of RBE for humans exposed to ionizing radiation in the nuclear battlefield or other potential future military operations.

Extend microdosimetry calculations and measurements to develop new models of radiation damage, leading to a better understanding of damage mechanisms.

Accomplishments

We have completed a major upgrade and verification of AFRRI radiation dosimetry systems through close collaboration with the National Bureau of Standards and other laboratories.

For neutron dosimetry of the AFRRI TRIGA reactor, radiation field characterizations in close agreement have been obtained through calculations of neutron and gamma-ray energy spectra and fluence compared to experimental measurements of kerma by ionization chambers, fluence by activation foils, and dose by calorimetry.

For photon-electron dosimetry of the AFRRI LINAC and cobalt-60 facility, ionization chamber measurements of dose intercompared with NBS have also been verified by ferrous sulfate dosimetry. Cross referencing with calorimetry dosimetry is experimentally complete but not yet analyzed.

Neutron energy spectra and calculated dose distributions have been obtained for a monkey phantom irradiated in TRIGA reactor radiation fields.

Neutron and gamma dose distributions have been measured in two canine phantoms.

An up-to-date set of calculations has been completed defining the radiation fields that may exist in potential nuclear battlefields.

RADIATION SCIENCES DEPARTMENT

Group Title: Nuclear Sciences Division

Objectives

To investigate the mechanisms and pathogenesis of ionizing radiation-induced structural and functional changes in various organ systems that are involved in radiation-induced alteration of their morphology and homeostatic function. This research is directly related to the problem of the combat performance decrement in both acute and delayed radiation syndromes. Research includes studies of morphological and functional alterations of cardiovascular, gastrointestinal, hepatobiliary, renal, and reticuloendothelial systems, as well as structural and functional changes in neuroreceptors in the brain and other parenchymal organs in which both adrenergic and cholinergic receptors are altered by the exposure to ionizing radiation. The studies will utilize both in vitro and in vivo nuclear medicine techniques including planar scintigraphy and single-photon emission tomographic reconstruction imaging, tissue distribution studies, and quantitative autoradiography.

Current Status

Research on radiation-induced changes of the cholinergic neuroreceptors is 10% complete.

Cardiovascular studies involving precordial irradiation with 30, 60, and 100 Gy on the canine model are completed. The results have demonstrated time- and dose-dependent decrements of the ejection fraction of the heart after single exposure of the precordial region to gamma radiation.

Studies of gastric emptying of solid and liquid contents after ionizing radiation are approximately 30% complete. Mechanisms of emesis and gastric reflux are in the initial phase of investigation. Gall bladder ejection fraction evaluations and biliary duodenogastric reflux studies are also in the initial experimental phase. The influence of sedative, narcotic, and hypnotic drugs on the hepatobiliary kinetics is 25% complete.

Future Goals and Directions

Continue research on the radiation-induced changes of the cardiovascular system by the experimental model of early detection of structural and functional cardiac alteration in the latent asymptomatic phase where present methods of stroke volume and ejection fraction determinations are of insufficient sensitivity. These early changes will be investigated by the methods of metabolic functions of the heart using radiolabeled fatty acids and glucose analogs and by the use of single photon and positron emission tomography. These studies are planned as a collaborative effort between AFRRI, Brookhaven National Laboratory, and Massachusetts General Hospital Department of Nuclear Medicine. Continue research on the radiation-induced changes of the cholinergic receptors in the brain and parenchymal organs by the use of biodistribution and quantitative autoradiography studies. Continue research on the etiology and pathogenesis of radiation-induced vomiting. These studies will include collaboration with the University of New Hampshire, where the method of surgical removal of area

postrema is being routinely performed in the feline experimental model and will be used in a primate model at AFRRI. Gastric motility, electrical activity, liquid, and solid-meal emptying response to various pharmacologic and hormonal agents and gastroesophageal reflux studies will continue. Hepatobiliary research will continue to address the effects of pharmacologic and hormonal agents, as well as the effects of gamma and neutron irradiation on the hepatobiliary kinetics and the role of duodenogastric bile reflux in radiation-induced nausea and vomiting. Research on the metabolic pathways of transuranic elements as a consequence of the delayed effect of nuclear weapons, radiation toxicology, and therapeutic removal of actinides is vital mission-related research planned to be conducted in the Nuclear Sciences Division. Blood flow determination of the central nervous system and its role in acute transient incapacitation will be addressed by noninvasive imaging methods of Xe-133 and Kr-81 flow imaging and by the use of radiolabeled microspheres. The reticuloendothelial system will be studied by the use of In-111, In-113m, and Tc-99m microcolloids in the experimental work on RES response to radiation and combined injury. Bone healing after radiation and traumatic injury will be studied by the use of planar scintigraphy, autoradiography, and ultrastructure after ultrasonic stimulation.

Accomplishments

Gastrointestinal studies on radiation-induced vomiting in primates, suppression of radiation-induced vomiting by pharmacologic agents, effects of endorphins and prostaglandins on gastric function, radiation-induced changes of gastric electrical activity, and studies on the morphological changes of gastric mucosa after ionizing radiation.

Bone healing studies after radiation and trauma studied by planar scintigraphy.

Cholinergic receptor studies in different parts of the central nervous system and various parenchymal organs have resulted in original observations of photon and neutron changes in muscarinic receptors after irradiation.

Cardiovascular studies have resulted in an original contribution to the understanding of symptomatology, etiology, and pathogenesis of radiation-induced alterations of the heart, which was found to be dose- and time-dependent and determined latent and sudden death phases in the various time intervals after irradiation.

Hepatobiliary studies of the effects of gamma radiation on intrahepatic and extrahepatic biliary kinetics have been recognized and awarded prizes as the first contribution to the literature of facilitated choleresis in postirradiation syndrome which is time- and dose-dependent. The effects of hypnotic agents have been an original contribution to the field of hepatobiliary kinetics in radiation diseases.

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